580

Vol. 19 No. 5

May, 1953

THE BOTANICAL REVIEW

Interpreting Botanical Progress

Antibiotics Produced by Actinomycetes ... R. G. BENEDICT 229

THE JOHN CRERAR LIBRARY

RECEIVED
MAY 13 1953

FUTURE CONTENTS
ON INSIDE BACK COVER

Published Monthly except August and September at
111 E. Chestnut St., Lancaster, Pa.

Entered as second-class mail matter February 4, 1935, at the Post Office at Lancaster, Pennsylvania, under the Act of March 3, 1879.

THE BOTANICAL REVIEW

Founded in 1935 by
HENRY ALLAN GLEASON and EDMUND H. FULLING
Managed, edited and published at
The New York Botanical Garden
by
EDMUND H. FULLING

Advisory Editors

PROF. CHESTER A. ARNOLD
University of Michigan

PROF. MYRON P. BACKUS
University of Wisconsin

PROF. HERBERT C. HANSON
Catholic University of America

PROF. M. M. RHOADES
University of Illinois

PROF. GILBERT SMITH
Stanford University

PROF. RUSSEL B. STEVENS
University of Tennessee

PROF. S. F. TRELEASE
Columbia University

PROF. CONWAY ZIRKLE
University of Pennsylvania

THE BOTANICAL REVIEW is published monthly except August and September. Subscription rate for all countries is $6.00; single copies, 60¢. All subscriptions are payable in advance at par in Lancaster, Pa., or New York. Checks should be made payable to THE BOTANICAL REVIEW.

Articles are obtained primarily by invitation. Unsolicited manuscripts are also welcome. In all cases, however, the editor reserves the right to accept, reject or suggest revisions in submitted material. All manuscripts should be on double-space typewritten pages, with references and citations in accordance with the customary style of THE BOTANICAL REVIEW.

Missing numbers can not be supplied unless notice is received within two months after appearance of issues concerned.

All correspondence should be addressed to THE BOTANICAL REVIEW, at 111 E. Chestnut St., Lancaster, Pa., or Fordham P. O., New York, N. Y.

THE BOTANICAL REVIEW

VOL. XIX MAY, 1953 No. 5

ANTIBIOTICS PRODUCED BY ACTINOMYCETES

R. G. BENEDICT

Northern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, U. S. Department of Agriculture, Peoria, Illinois

Introduction 229
Antibiotic Production Among the Actinomycetes 232
Antagonism Among Described Species of *Actinomyces, Streptomyces, Nocardia* and *Micromonospora* 234
Actinomycetous Antibiotics 238
Appendix to the Alphabetical List 291
Classification of Actinomycetous Antibiotics 292
Antibiotics and Nutrition 292
Discussion 302
Summary 303
Acknowledgments 305
References 306

INTRODUCTION

The screening of actinomycetes for antibiotic production has increased markedly within the past decade. Untold thousands of these organisms have been tested for this purpose. The initial success of penicillin resulted in great emphasis being placed on the screening of saprophytic molds during the years 1940–1945. Interest in the actinomycetes was renewed and given added impetus with the discovery of streptomycin. This interest was further enhanced by the failure to find outstanding and medicinally attractive low-toxicity factors from the higher fungi or the lower bacteria. Concentration of attention on the actinomycetes in screening programs has paid handsome dividends. The addition of Chloromycetin, aureomycin and terramycin to our medical armamentarium has markedly increased the number of infectious diseases which may be successfully treated with antibiotics. Furthermore, the discovery of these agents has stimulated the hope that other antagonistic strains, yet undiscovered among the actinomycetes, may provide additional useful tools from the standpoint of medicine, control of certain plant pathogens, and the like.

Brian (21) has pointed out in his excellent review of antibiotics produced by fungi that, unfortunately, in a majority of cases the fungi have not been adequately identified. The same statement is true to an appreciable extent when applied to antagonistic actinomycetes. A careful review of all pertinent literature available to the writer reveals that 115 antibiotics have been reported to be produced by the actinomycetes. Of this total number, 16 named factors appear to be synonymous with previously described antibiotics; five are unnamed and five are produced by chemical manipulations of known factors. Twenty-nine antibiotics were recovered from fermentations run on unidentified actinomycetes; 30 are reported from cultures thought to be identical with described species listed in the Bergey Manual of Determinative Bacteriology, Sixth Edition (*20a*); three are from organisms related to described species; 25 from new species not listed in the Bergey Manual; and two are from both "old" and "new" species.

Confronted with such figures, the reviewer is forced to conclude that two possibilities may account for the large number of antibiotics from unidentified actinomycetes: (*a*) that workers in the field lost all interest in their culture(s) when the product or products were found to be too toxic or failed in other respects as suitable medicinals; or (*b*) that the present system of classification of the actinomycetes, which is based on morphology and relatively few physiological tests, especially for the genus *Streptomyces*, makes it extremely difficult for competent investigators to "key down" an unknown culture. This opinion appears to be shared, in part, by Routien and Finlay (187), and a comprehensive discussion of the problem is found in a paper by Lindenbein (*142c*).

The morphological and physiological characteristics of a number of *Streptomyces* species are based on studies conducted on one or at most a limited number of isolates. Smith et al. (194), in their excellent monograph on the mesophilic spore-forming bacteria, emphasize the inadequacy of systems of classification in microorganisms which are not based upon examination of many isolates and on intelligent application of all available criteria.

Numerous complaints have been made to us regarding the difficulty of classifying *Streptomyces* cultures which have been isolated in antibiotic screening programs. The problem of proper identification is of considerable importance to investigators who apply for

process or product patents. The U. S. Patent Office requires that prior to issuance of a patent, a culture of the microorganism involved, identified as to genus and species, must be deposited with a bona fide culture collection in the United States. It is hoped that someone in the near future will approach the problem of reorganizing the genus *Streptomyces,* combining careful interpretation of cultural characteristics with new biochemical aids similar to those suggested by Pridham and Gottlieb (172).

The need for such a study is emphasized by the current large-scale production of the important medicinal antibiotics—streptomycin, Chloromycetin, aureomycin, terramycin, vitamin B_{12}—by members of this genus. In his editorial comments on the advent of "Ilotycin", Welsh (*247a*) points out that during May, 1952, the total production of the broad spectrum antibiotics—chloramphenicol, aureomycin and terramycin—had reached the astounding figure of 24 tons per month. During the same period 21 tons of streptomycin and dihydrostreptomycin and 30 tons of crystalline penicillin were produced. If we consider the figures cited for this one month as one-twelfth of the total tonnage for the year, the approximate wholesale values of the four *Streptomyces* antibiotics listed above and vitamin B_{12} for the year 1952 would be close to $225,000,000.

In the first section of this review the numbers of antagonistic strains of unidentified forms of *Actinomyces, Nocardia, Streptomyces* and *Micromonospora* occurring in random samples of soil, manure, composts and the like are considered. Following this, the antagonistic properties of species of each of the four genera are considered.

The second section includes brief characterizations of each isolated antibiotic, based on its more important biological, physical and chemical properties. An effort has been made to cover the published literature as well as to obtain papers "in press" and some additional information for which publication had not been envisioned. The literature citations are far from complete but are believed to represent an adequate coverage on most aspects pertinent to the review. An arbitrary key has been devised, mainly on the basis of chemical groupings, which attempts to separate the antibiotics derived from the actinomycetes. The publication early in 1952 of Raper's (175) comprehensive review obviates the need

for a discussion of the current application and importance of streptomycin, Chloromycetin, aureomycin and terramycin in medicine. Limited consideration will be given to the increasingly important use of vitamin B_{12} and antibiotic supplements in the feeding of nonruminant animals. Structural formulae of pure or crystalline antibiotic compounds of actinomycetous origin are shown insofar as they are known. The possible modes of action of the antibiotics are considered beyond the scope of this review, and for information on this and related aspects of the subject, the reader is referred to the recent reviews by Brownlee (31*a*), Kirby (121), Lumb (143*a*) and Work (251*a*).

ANTIBIOTIC PRODUCTION AMONG THE ACTINOMYCETES

The earliest studies on the distribution of antagonistic properties among soil actinomycetes were those of Nakhimovskaia (160) and Krassilnikov and Koreniako (124). The former work was done with only 80 strains, the latter with more than 1000 cultures isolated from soils. The most active strains were believed to represent several species of *Actinomyces,* but the single antagonistic factor ascribed to these organisms was said to resemble lysozyme. However, it differed from lysozyme in that it lysed staphylococci most easily and had no effect on *Micrococcus lysodeikticus.* Kriss (125) attempted to isolate the actinomyces lysozyme of Krassilnikov and Koreniako but was unsuccessful in eluting the agent from materials on which it had been absorbed. He reported it to have lytic action on many species of bacteria, both living and dead.

The earliest comprehensive survey of antagonistic properties of actinomycetes made in the United States was that of Waksman et al. (232). Among other extensive surveys of this nature are those of Welsch (246), Landerkin and Lochhead (132), Emerson (62), Meredith (152), Burkholder (35), Landerkin et al. (133), Rouatt et al. (186), Cercos and Rodriguez (45) and Kuroya et al. (130*a*). Their findings are summarized in Table I. Rouatt et al. (186) concluded that between 15% and 60% of all the cultures they isolated from natural substrates showed varying degrees of activity against different bacteria. Their findings suggest that the nature of the substrate has some bearing on the antagonistic properties of the actinomycetes found therein; those from decaying wood were the least active, whereas those obtained from the rhizosphere

of growing plants were most active. In contrast, Landerkin et al. (133) found that, although the numbers of organisms increase within the rhizosphere of clover, flax, wheat and potatoes as compared to control soil, the percentage of antagonistic actinomycetes decreases. They suggest that the relatively high percentage of antagonistic forms which they found in soils from northern regions may be related to the sparser vegetation and to the greater proportion of time during which no plant development occurs. The reader

TABLE I

SURVEYS OF ANTAGONISTIC ACTINOMYCETES ISOLATED FROM SOILS AND OTHER NATURAL SUBSTRATA

Investigators	No. of cultures tested	Percent of total active against			
		Gram-pos.	Gram-neg.	Acid-fast	Higher fungi
Nakhimovskaia (160)	80	59	—	—	—
Krassilnikov and Koreniako (124)	1,000	?	—	—	—
Waksman et al. (232)	244	43 **	—	—	—
Welsch (246)	164	46 **	—	—	—
Meredith (152)	7,642	—	—	—	1.2 ***
Burkholder (35)	7,369	25 *	3 †	—	7 ‡
Landerkin and Lochhead (132)	591	71	—	—	—
Landerkin et al. (133)	660	48	8.2	19.5	20.9
Emerson et al. (62)	772	52 *+	—	—	47
Rouatt et al. (186)	544	39	10	—	—
Cercos and Rodriguez (45)	54	90 **	20+	60	20
Kuroya et al. (130*a*)	1,223	13 *	—	—	—

* Against *M. pyogenes* var. *aureus*, only.
† Against *Escherichia coli*, only.
‡ Against *Candida albicans*, only.
** Against *Bacillus subtilis*, only.
*** Against *Fusarium oxysporum cubense*, only.
*+ Against Gram-positive and Gram-negative types.

is referred to the interesting and comprehensive review by Routien and Finlay (187) on problems encountered in the search for antibiotic-producing microorganisms.

Unnumbered thousands of actinomycetes isolated from soils collected from many countries have been screened for antibiotic potentialities by workers in governmental and university laboratories, and especially by companies engaged in the manufacture of pharmaceuticals, biologics and fermentation products. No data are

available on the percentage of antagonistic and inactive strains from many of these surveys. Most of the screening results found in the literature are based on various modifications of the technique of Waksman et al. (232), wherein the actinomycete is first inoculated on various agar media and allowed to incubate for a specified time. Next, cross streaks are made with several bacteria, including selected Gram-positive, Gram-negative, saprophytic acid-fast forms and some higher fungi. The plates are then incubated at the optimum temperature of the test organisms and examined for growth inhibition. However, Bennett (14) has suggested that some actinomycetes which do not show agar cross streak inhibition may produce antibiotically active broths when tested in shaken-flask cultures, and some screening programs are based entirely upon antibiotic activity observed after purified isolates are shaken in selected media.

ANTAGONISM AMONG DESCRIBED SPECIES OF ACTINOMYCES, STREPTOMYCES, NOCARDIA AND MICROMONOSPORA

Although the order Actinomycetales includes the family Mycobacteriaceae, members of this family will not be considered in this review.

Actinomyces Harz. The Bergey Manual of Determinative Bacteriology, Sixth Edition, lists only two well-described species of *Actinomyces, A. bovis* and *A. israeli.* The reviewer failed to find any report in the literature wherein strains of either of these species had been found to be antagonistic to other microorganisms.

Streptomyces Waksman and Henrici. This genus is the largest and the most important one, antibiotically speaking, of the order Actinomycetales. There are 73 defined species listed in Bergey's Manual, and its appendix includes many additional named species whose descriptions are inadequate. From 1948 to November, 1952, 28 antibiotically active proposed new species were described. The following facts have been brought forth in numerous articles regarding antibiotic production by various species of *Streptomyces:* (*a*) strains of a particular species may vary markedly in their antibiotic-producing capacity, ranging from those which are very active to those producing none; (*b*) no universally accepted standards exist for determining whether a culture is active or inactive; and (*c*) a number of antibiotically active strains are unidentified, or

TABLE II

ANTIBIOTICS PRODUCED BY IDENTIFIED STRAINS OF *Streptomyces*

Species recognized in Bergey Manual, 6th Ed.		New species not listed in Bergey Manual	
Species	Antibiotic(s)	Species	Antibiotic(s)
albus	Thiolutin Actinomycetin	*chrysomallus*	Actinochrysin, originally called, actinomycin C
californicus	Viomycin		
coelicolor	Coelicolorin Mycetin Actinomyces lysozyme	*aureofaciens*	Aureomycin
		rochei	Borrelidin
		venezuelae *omiyaensis*	Chloromycetin *
roseochromogenus	Roseomycin	*roseoflavus*	Flavomycin Mycelin
parvus	Actinomycin	*griseoluteus*	Griseolutein
flavus	Actinomycin Flavacid	*griseocarneus*	Hydroxystreptomycin Rotaventin
antibioticus	Actinomycin		
flaveolus	Actinomycin	*netropsis*	Netropsin
flavovirens	Actinomycin	*nitrosporeus*	Nitrosporin
aureus	Luteomycin Fungicidin RAW Anti-phlei factor	*rimosus*	Terramycin Rimocidin *
		bikiniensis	Streptomycin
lavendulae	Streptothricin complex Ehrlichin	*vinaceus*	Vinactins A, B, and C
		virginiae	Actithiazic acid
rubrireticuli (variants)	Reticulin Rotaventin Streptin	*resistomycificus*	Resistomycin
		cinnamoneus	Cinnamycin
cacaoi	Cacaomycetin	*purpurascens*	Rhodomycins A and B
griseus	Streptomycin Mannosidostreptomycin Acti-dione * Streptocin Rhodomycetin Grisein Grisein-like	*albo-niger*	Achromycin
		felleus	Picromycin
		endus	Endomycin
		canescus	Ascosin
		abikoensum	Abikoviromycin
		hachijoensis	Trichomycin
erythreus	"Ilotycin" *	*floridae*	Viomycin
halstedii	"Magnamycin" *		
griseoflavus or *alboflavus*	Terramycin Rimocidin *		
fradiae	Neomycin(s) Fradicin		
thermophilus	Thermomycin		
rubescens	Abikoviromycin		

* Trademark.

reported as variants of two or more species. Therefore the reviewer's data on strains reported as not tested or inactive probably contain some species which are active but whose identity has not been established.

Table II lists active strains of identified *Streptomyces* reported in the literature and the antibiotics which have been isolated from their fermentation liquors or mycelia.

The antibiotic activity, so far as known, of the remaining described *Streptomyces* species from the Bergey Manual is shown in Table III. Included are 40 species selected from the Northern Regional Laboratory Culture Collection which were screened by Benedict and Lindenfelser (10) for antibacterial or antifungal activity by a cross streak method.

The following species were not available for testing, and many of these may be lost, since they are not listed in well-known culture collections: *Streptomyces malenconi, diastatochromogenes, flavochromogenes, fulvissimus, lieski, flocculus, melanosporeus, acidophilus, thermofuscus, fordii, gallicus, pelletieri, listeri, upcottii, hortonensis, beddardii, kimberi, somaliensis* and *panjae*. No reference was made to possible antibiotic activity of a recently reported new species, *S. nitrificans* (Isenberg et al., 109), or that of *S. salmonicida*, isolated from blueblack salmon by Rucker (187*b*).

Nocardia Trevisan. Although 33 well-defined species and a large number of incompletely described species of this genus are listed in the Bergey Manual, the number of antibiotics which have been isolated is very small. The reasons may be twofold: (*a*) on the whole, species of *Nocardia* may be generally less antagonistic than the *Streptomyces* group, or (*b*) organisms of this type may often be bypassed in screening, since the whole mycelium tends to break up, within a relatively short time, into regularly cylindrical short cells and then into coccoid cells. Thus in the isolation of *Streptomyces*-like forms, colonies of *Nocardia* in the latter stages of the growth cycle may be ignored because of their bacteria-like form. A search of the literature indicates that, to date, seven identified and four unidentified species of *Nocardia* are known to produce antibiotics. These are *N. acidophilus*, which synthesizes mycomycin (40); *Proactinomyces cyaneus-antibioticus*, which produces litmocidin (76); *N. coeliaca*, which forms nocardin (63); *N. gardneri*, which forms proactinomycins A, B and C (74); and *N. flavescens*, from which

Stoll et al. (199) isolated nocardamin. Also, Gardner and Chain (74) have reported that *Proactinomyces polychromogenes* is antagonistic toward Gram-positive cocci; Ueda and Uesaka (214*a*) have recently reported the isolation of neonocardin from *N. kuroishi*. Bick et al. (15*b*) have isolated nocardianin from an unidentified

TABLE III

ANTIBIOTIC ACTIVITY OF SPECIES OF *Streptomyces* TESTED AT THE NORTHERN REGIONAL RESEARCH LABORATORY AND ELSEWHERE

Species	Active	Inactive	Species	Active	Inactive
africanus	+		*ipomoea*	+	
albidoflavus	+		*limosus* **	+	
alboflavus	+		*lipmanii* *		+
albosporeus	+		*melanocyclus* *		+
alvei	+		*microflavus* *	+	
annulatus *	+ (A)		*novaecaesareae*		+
bobilae	+		*odorifera*	+	
carneus	+		*olivaceus* *	+	
cellulosae	+		*olivocromogenus* *	+	
citreus		+	*phaeochromogenus* *	+	
collinus **	+		*poolensis* *	+	
diastaticus	+		*purpeochromogenus*	+	
erythreus *	+		*ruber* *	+	
erythrochromogenus *	+		*rutgersensis* *	+	
exfoliatus	+		*scabies* *	+	
flavovirens *	+		*sulphureus*	+	
gelaticus		+	*verne* *	+	
gibsonii	+		*violaceoniger* *	+	
gougeroti *	+ (A)		*viridis*	+	
griseolus		+	*viridochromogenus* *	+ (A)	
halstedii *	+		*willmorei*	+	
hygroscopicus	+		*xanthophaeus* **	+	
intermedius	+				

* Species previously tested and reported active in literature (117*a*, 229*a*, 4*a*, 150*a* and 147*c*) except for *S. melanocyclus* which was reported negative (124).

+ (A) Antifungal activity, only.

** Nature of antibiotic(s) not yet reported.

species of *Nocardia*, and Machlowitz et al. (144*a*) have reported cardicin from three unidentified species of *Nocardia*.

Micromonospora Orskov. Members of this genus occur mainly in composted manure, in dust, soil and lake bottoms. Many, being thermophilic, can grow at 65°. Five species are described in the Bergey Manual, and four antibiotic factors have been reported in

the literature. The strain which produces the antibiotic micromonosporin (Waksman et al., 230) was not identified as to species. A recent account of an actinomycin identical with that of Waksman and Tishler from *Micromonospora* was that of Fisher et al. (70). The strain producing this factor was similar to *M. globosa,* and it is the first instance wherein an identical agent has been reported from species of two genera of the Actinomycetales. Taira and Fujii (205*c*) isolated microcin A and B from an unidentified form of *Micromonospora.*

ACTINOMYCETOUS ANTIBIOTICS

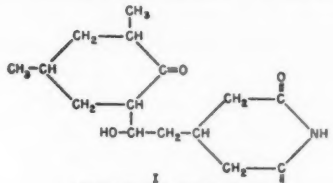
The antibiotics thus far reported from actinomycetes are briefly characterized in this section in alphabetical order. Space does not permit detailed chemical data, but the more important biological, pharmacological, chemical and physical properties are included. Some data on stability and toxicity are also reported. The chemical complexity of many antibiotics in this group probably accounts for the small number of structural formulae which have been worked out and which are shown in Table IV. In each instance, insofar as is known, the species of *Streptomyces, Nocardia* or *Micromonospora* which produces a given antibiotic is listed.

Recent information supplied by Maeda (145*a*) on the close similarity of flavomycin, dextromycin and streptothricins BI and BII to the neomycin complex would suggest that these substances and catenulin be discussed under a general heading entitled "Neomycin-like substances". Rather than break the alphabetical continuity, the reviewer chose to keep them in their normal alphabetical order and to group them together in the arbitrary classification scheme.

ABIKOVIROMYCIN. According to Umezawa et al. (222), abikoviromycin may be extracted from culture liquors with ethyl acetate and gives a characteristic red color upon five minutes heating at 100° in 1% phosphate buffer at pH 7.0. The substance gives positive Molisch, Tollen's and silver mirror tests and sublimes during freeze drying. It is readily decomposed by heat, acid or exposure in a dry state. Loss of activity is denoted by the same change in color as that already recorded for heating in phosphate buffer. It is weakly antibacterial and antifungal. It exerts a protective effect for animals at 1–8,000,000 dilution when mixed with either

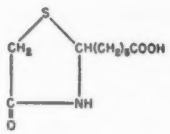
TABLE IV

Structural Formulae of Actinomycetous Antibiotics



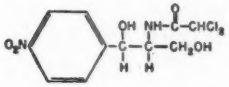
I

Acti-dione (cycloheximide)



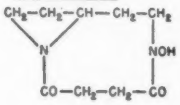
II

Actithiozic Acid



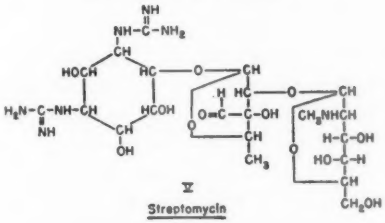
III

Chloramphenicol (Chloromycetin)



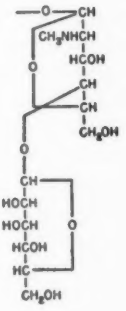
IV

Nocardamin



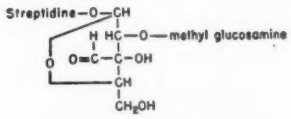
V

Streptomycin



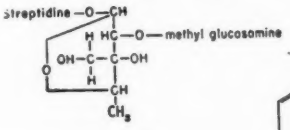
VII

Mannosidostreptomycin
(streptidine and streptose portions not shown)



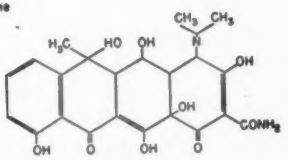
VI

Hydroxystreptomycin



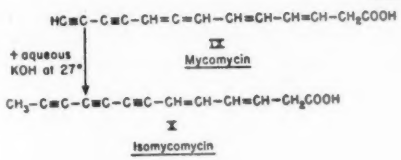
VIII

Dihydrostreptomycin



XI

Terramycin



IX

Mycomycin

+ aqueous KOH at 27°

X

Isomycomycin

eastern or western encephalomyelitis virus and injected intracerebrally into mice.

The intravenous and subcutaneous LD_{50} doses in 12-gm. mice are approximately 0.1 mg. and 1 mg., respectively. Two *Streptomyces* species were reported to produce abikoviromycin and were identified as *S. rubescens* and *S. abikoensum,* n. sp. (222).

ACHROMYCIN. According to Porter et al. (171*b*), achromycin, so named because it is devoid of pigment in the crystalline state, has been crystallized in the form of various acid salts and as the free base. The base melts at 175.5–177° (uncorrected), and analyses indicate C, 56.55%; H, 6.35%; and N, 20.88. No evidence of the presence of halogen, sulfur, phosphorous or metallic elements could be found. The antibiotic exhibits characteristic infra red absorption at 25 different wave lengths.

It is quite active against Gram-positive rods and cocci and against *Klebsiella pneumoniae.* Less activity is shown against *M. tuberculosis* 607 and only weak activity against many Gram-negative rods. Achromycin shows curative effects against *Trypanosoma equiperdum* in mice when administered orally or parenterally. Preliminary acute toxicity tests in mice showed LD_{50} values of approximately 350 mg./kg. intravenously, 525 mg./kg. intraperitoneally, and 675 mg./kg. for oral administrations. The achromycin-producing strain has been assigned the name *Streptomyces albo-niger* (171*b*).

ACTI-DIONE * (cycloheximide) ($C_{14}H_{23}NO_4$). Recrystallization from water yielded colorless plates, m.p. 119.5–121°, which are soluble in water, chloroform and ether, and are thermostable and optically active, $[\alpha]_D^{29} = -3.88°$ (C = 9.47 ethanol). Originally discovered by Whiffen et al. (248*a*), it is active against various yeasts and certain higher fungi. Acti-dione has given promise in the control of certain smuts and fungi on seeds and fungus diseases of plants when administered either as a dust or as a liquid (Henry et al., 95; Vaughn et al., 226; Vaughn and Hamner, 225). *Saccharomyces pastorianus* increased in resistance from inhibitory concentration of 0.06 μg./ml. to 4.08 μg./ml. in three transfers. Three derivatives were prepared, the semicarbazone, acetate and oxime; the first had about one-twentieth the activity of Acti-dione, whereas the other

* Trademark name.

two were inactive (Kornfeld et al., 123). The crude product is highly irritating to skin, caused vomiting in dogs and nausea in humans. The LD_{50} (mg./kg.) dose for mice is 150, but the figure for rats tested by the same route is only 2.5 (Goth and Robinson, 75*a*). Thus far, Acti-dione has been produced only by certain strains of *S. griseus*. It has been recovered from streptomycin-forming strains and from a streptocin-producing strain, Waksman's No. 3533 (Kupferberg et al., 127), grown in the Acti-dione medium of Whiffen (248). An Acti-dione-like substance was reported as a product of the fungicidin-producing strain of Hazen and Brown (93).

ACTINOCHRYSIN (Actinomycin C) ($C_{40}H_{57}O_{11}N_7$). Brockmann and Grubhofer (23) found that it crystallizes from ethyl acetate in the form of alizarine-red bipyramids, m.p. 254°, with decomposition (corrected). It is readily soluble in acetone, chloroform and benzene, sparingly in alcohol and ethyl acetate, and only very sparingly in water. Specific rotation in 0.25% methanolic solution, $[\alpha]_D^{17} = -310 \pm 3°$. The actinochrysin molecule appears to consist of a chromophoric system termed " despeptidoactinomycin C " which crystallizes as dark red needles; it may be a dimethyl-trioxy-aminoanthraquinone ($C_{16}H_{13}O_5N$) to which is attached the amino acids L-threonine, D-valine, sarcosine, D-alloisoleucine, L-proline and N-methyl-L-valine. The absence of free carboxyl and amino groups suggests a cyclic peptide structure. The antibiotic inhibited the growth of *Micrococcus pyogenes* var. *aureus* at 1:20 million, *B. subtilis* at 1:100 million, and *Escherichia coli* at 1:10,000. Mice succumb within 24 hours after receiving 50 mg./kg. orally or five mg./kg. intraperitoneally. The culture which produces the antibiotic has been named *Streptomyces chrysomallus* (24, 25).

ACTINOFLAVIN. Umezawa et al. (217) isolated and crystallized this antibiotic from a culture of *Streptomyces flavus*. They later concluded that the antibiotic is identical with Waksman's actinomycin A. Hirata and Nakanishi (99) reexamined Umezawa's material and found slight differences in the reported absorption band and carbon content. However, they indicated that the two substances, actinoflavin and actinomycin, are very similar and probably possess almost identical structures. Therefore it is the reviewer's opinion that actinoflavin as a chemical entity must await

further structural proof before being considered a new member of the actinomycin complex.

ACTINOMYCELINE. This factor is described by Cercos (44) as a yellowish-green pigment of neutral reaction; very soluble in water and ethanol, showing intense fluorescence in the latter; soluble in methyl acetate, moderately in acetone, slightly in amyl acetate and chloroform; insoluble in diethyl ether and benzene. It is stable in aqueous solution at pH 7, less stable at pH 2.0, and is destroyed in .1 *N* NaOH after 24 hours. More than 50% of its activity is lost on boiling in aqueous solution for 30 minutes. It is most effective against Gram-positive organisms and exerts no activity against mycobacteria or higher fungi. Subcutaneous injections of 25 mg./kg. in rats caused death in all cases. The culture which produces the antibiotic is believed to be closely related to *Streptomyces antibioticus* (44).

ACTINOMYCES LYSOZYME (See Mycetin and Actinomycetin). A lytic agent first reported by Krassilnikov and Koreniako (124) and further investigated by Kriss (125). According to Welsch (246), it differs from the lytic agent of actinomycetin by its relative thermostability, resistance to ultraviolet radiations, easy adsorption on various substrates, and abundant production in synthetic media. It is similar to the lytic principle of actinomycetin by virtue of its solubility in water, insolubility in organic solvents, and lower activity in acid media. A comparison of the properties of mycetin and actinomyces lysozyme, both of which are produced by *Actinomyces violaceus,* reveals marked similarities. The major point of difference lies in the solubility of the two substances in organic solvents. Actinomyces lysozyme is said to be insoluble in solvents, whereas mycetin is soluble in benzene and chloroform. In the reviewer's opinion the introductory statement in the discussion of actinomycetin applies equally well to actinomyces lysozyme.

ACTINOMYCETIN. The status of this substance or substances as an antibiotic is not clear. Studies by Welsch (247) indicate that actinomycetin contains one or more antibiotic agents which have a lytic effect upon certain living bacteria. Earlier work (245) suggested that the lytic action of actinomycetin is twofold: first, that susceptible cells are killed by a selectively bactericidal lipoid; second, that the dead cells are then dissolved by the bacteriolytic enzyme, which alone is responsible for the lysis of heat-killed bac-

teria. To the reviewer's knowledge, few data have been released on chemical or physical properties of crude actinomycetin. It is reported to be soluble in water and insoluble in organic solvents; stable at room temperature but unstable at pH 4.0. Repeated precipitation with ammonium sulfate yielded a tenfold increase in activity. It is produced by the " C " strain of *Streptomyces albus* (247).

ACTINOMYCIN COMPLEX

ACTINOMYCIN ($C_{41}H_{56}N_8O_{11}$). Waksman and Woodruff (238, 239) found that actinomycin, the first antibiotic to be isolated in a pure state from an actinomycete, crystallizes from ethyl acetate or from acetone-ether mixtures as red platelets, m.p. 250°, $[\alpha]_D^{25}$ (C = 0.25% in ethanol) $-320 \pm 5°$. It is soluble in chloroform, acetone, ethanol, hot ethyl acetate, carbon disulfide and benzene, but only slightly soluble in water or ether. It is stable in aqueous alcohol when boiled for 30 minutes, but unstable under these conditions if dispensed in dilute acid or alkali. An alcoholic solution of actinomycin gives no coloration with ferric chloride; it shows characteristic light absorption in the visible region ($E^{1\%}_{1\text{ cm.}} = 200$ at 450 mμ in alcohol) and in the ultraviolet region ($E^{1\%}_{1\text{ cm.}} = 216$ at 215 mμ). Like actinochrysin, it is powerfully bacteriostatic against certain Gram-positive forms. Fungistatic activity is reported against a variety of higher fungi. Waksman and Tishler (237) found that ten μg. given intraperitoneally or subcutaneously killed 20-gram mice in 24 to 48 hours; oral administration was also highly toxic (236).

Lehr and Berger (140) isolated a crystalline actinomycin-like antibiotic which was designated " antibiotic X-45 ". Samples of this material were critically examined by Dalgleish and Todd (48), later by Dalgleish et al. (50). Antibiotic X-45 was assigned the empirical formula $C_{41}H_{58}O_{11}N_8$ instead of $C_{41}H_{54}O_{12}N_8$ as formerly reported, thus removing the major point of difference between Waksman and Tishler's actinomycin and " antibiotic X-45 ". Five amino acids were crystallized from the hydrolysis of X-45 and were identified as D-valine, L-proline, threonine, N-methylvaline and sarcosine. Thus the only apparent difference between actinomycin and actinochrysin (actinomycin C) is that the latter contains D-alloisoleucine plus the amino acids shown above.

Actinomycin is produced in culture by *Streptomyces antibioticus*

and by *Streptomyces* S-4 and 36-G (231). Trussell and Richardson (214) isolated a substance identified chemically and biologically as actinomycin from *Streptomyces* A9-6, which was different from any of the above three strains. Kocholaty et al. (122) recovered actinomycin from a culture tentatively identified as *Streptomyces parvus*. Recently Fisher et al. (70) reported the isolation of actinomycin from a form of *Micromonospora* which is closely related to *M. globosa*. Paper chromatographs of an acid hydrolyzate gave the same amino acids as shown by actinomycin and X-45. Umezawa et al. (215) and Kurosawa (128) reported the isolation of actinomycin from strains of *Streptomyces flaveolus*, and Pridham and Gottlieb (172) identified actinomycin-producing 83D as a strain of *S. flavovirens*. Benedict and Lindenfelser (10) isolated strains of six apparently distinct species of *Streptomyces* which produce actinomycin-like antibiotics. One strain appears to be identical with *Streptomyces parvus*, one resembles strain A9-6 of Trussell and Richardson, one closely resembles *Streptomyces flavus*, and two are similar to *S. flavovirens* or *S. flaveolus*. One strain is different from *S. antibioticus* and *S. chrysomallus*, but none of them has been compared with Waksman's S-4 or 36-G.

ACTINOMYCIN B. The original designation of a fraction from *S. antibioticus* liquors as actinomycin B (238) was later withdrawn by Waksman et al. (231) because the low activity of this fraction was thought to be due to traces of actinomycin A present as impurities. The substance " antibiotic X-45 ", provisionally referred to as actinomycin B by Dalgleish and Todd (49), and first reported as $C_{41}H_{54}O_{12}N_8$ (48), was later found to correspond to $C_{41}H_{58}O_{11}N_8$. Thus Dalgleish et al. (50) have practically eliminated all possibilities that X-45 and actinomycin are separate entities, and the designation, actinomycin B, for X-45 in the reviewer's opinion should be held in abeyance, unless further work proves that it is chemically distinct.

ACTINOMYCIN J_1 and J_2 (see Actinoflavin). Although Hirata and Nakanishi (99) originally concluded that actinomycin and actinoflavin are very similar, a later paper by these authors (100) pointed out that the position of the absorption maximum in 95% ethanol is 435 mμ compared with 450 mμ and 230–250 mμ obtained by Waksman for actinomycin. The difference in percentage of

carbon (55.45 as compared with Waksman's 59.01) prompted them to rename the substance "actinomycin J_1". Upon purification, the substance actinomycin J_2 was found to be devoid of antibiotic activity and was identified as the dodecyl ester of 5-ketostearic acid.

ACTINONE. The most potent preparation was obtained as a yellowish powder which is active in dilutions ranging from 1 : 1,000,000 to 1 : 1,500,000 against *Saccharomyces* sp. and *Trichophyton,* but not against other fungi or bacteria. Actinone is soluble in ether and butyl acetate; less soluble in benzene, petroleum ether and ethyl acetate; and insoluble in chloroform. The powder contained less than 1% nitrogen. Ten-gram mice were reported to survive doses of 1000 mg./kg. by subcutaneous and intravenous routes. It is produced by *Streptomyces* 89-C-2 (Ikeda et al., 108) which is closely related to *S. antibioticus.*

ACTINORHODINE ($C_{23}H_{20}O_{10}$). According to Brockmann and Pini (28), actinorhodine occurs as fine red needles, with no definite melting point, and it is soluble in pyridine, dioxane, tetrahydrofuran, and in alkali solution. The antibiotic is believed to possess a quinone structure, having three free hydroxyl groups, one carboxyl group and two hydroxyl groups adjacent to a carboxyl group. Ultraviolet maxima appear at 531 and 571 mμ in butanol. Shockman and Waksman (192) found that actinorhodine also gives ultraviolet maxima at 614 to 621 mμ, which tends to differentiate the former from a similar antibiotic, rhodomycetin. The antibiotic shows a blue color in alkaline solution, dark blue in sulfuric acid, and becomes red violet on the addition of boric acid. Growth of *Staphylococcus aureus* was inhibited at 1 : 100,000. Information on the growth-inhibiting effect on Gram-positive rods or Gram-negative organisms is lacking. The *Actinomyces* culture producing the antibiotic was not further identified.

ACTINORUBIN. Early studies on this antibiotic were made by Kelner et al. (120). It is reported to be a white hygroscopic base (116) which produces a crystalline derivative when treated with sodium helianthate. The assignment of $C_6H_{14}N_3O_2$ or $C_9H_{22}N_5O_4$ as the dihelianthate of a base, m.p. 206–214° (corrected, decomposition), is tentative. The antibiotic dialyses through cellophane and is stable to 15 minutes of boiling in aqueous solution at pH 6–7. It is active against Gram-positive and Gram-negative bac-

teria. The toxic dose (LD_{100}) for white mice, intraperitoneally, was 1.37 mg. (158). The chemical characteristics, reactions and antibacterial spectra suggest a strong resemblance to streptothricin. The reviewer is inclined to agree with Wintersteiner and Dutcher (250) that it is questionable that actinorubin and lavendulin are distinct entities closely related to but not identical with streptothricin, since this contention rests on certain discrepancies in the analytical composition of the helianthates. The reviewer has tested the actinorubin- and lavendulin-producing strains on spectrum plates with 12 test organisms, including a streptothricin-resistant strain of *B. subtilis,* and found that these spectra and those produced by streptothricin-forming strains of *S. lavendulae* were indistinguishable. Paper strip chromatograms of actinorubin and lavendulin crude beers were resolved in wet butanol-para toluene sulfonic acid. Four zones of inhibition appeared from each spotted strip, showing apparently identical R_f mobilities. The distance traveled by the major component of each corresponded to that shown by pure streptothricin. The antibiotic is produced in culture by *Actinomyces* A-105 which, according to Kelner and Morton (119), resembles in some respects *A. erythreus, fradii, albosporeus* and *californicus* without being unmistakably one, or a variant of any, of these species.

ACTITHIAZIC ACID ($C_9H_{15}O_3NS$). Chemically speaking, this compound is (-)2-(5-carboxypentyl)-4-thiazolidone (150*b*), or 4-thiazolidone-2-caproic acid (86). (See Table IV for the structural formula). Actithiazic acid is a crystalline analogue of biotin, according to Grundy et al. (86) and Schenk and DeRose (190). Independent investigations on the same antibiotic were made by Sobin (195*a*) and McLamore et al. (150*b*). Tejera et al. (207*b*) reported on a factor called " mycobacidin " which is also identical with actithiazic acid. Actithiazic acid melts at 140–141° and shows optical activity; $-60°$ in ethanol (190) and $[\alpha]_D^{23} - 54°$ (C 1, methanol) (150*b*). It is soluble in butanol and the lower alcohols, and the sodium salt is very soluble in water. Aqueous solutions of the antibiotic are quite stable over a wide pH range at room temperature. This interesting factor is specifically active against various mycobacteria. Weak activity was exerted against several non-acid fast types in a synthetic medium at 1 mg./ml. As little as 0.008 μg./ml. of biotin reversed the activity of actithiazic acid

against *Mycobacterium* 607. Clark and Schenk (46) synthesized the antibiotic and found that the DL form is only half as active as the L form. Hwang (107) found that mice can withstand injections of more than 1500 mg./kg. of the synthetic DL form. Five actithiazic acid-producing strains were carefully studied before assigning them a new species designation, *Streptomyces virginiae* (86). The mycobacidin strain of Tejera et al. (207*b*) was said to resemble *S. lavendulae* in many characteristics.

ANTIBIOTIC EI_5. In a search for new antibiotics effective against *Mycobacterium tuberculosis* Weiser et al. (244) isolated a factor, designated EI_5, which, like actinorubin, was soluble in water and acid methanol, insoluble in ether and acetone, and stable in aqueous solution when boiled. Comprehensive spectrum tests also indicated a similarity to actinorubin as did toxicity studies wherein white mice exhibited focal necrosis of the liver and renal epithelial damage after receiving large doses (150,000–270,000 units/kg.). The culture which produced this antibiotic was unidentified; it was reported to partially resemble *S. griseus* but to differ extensively from *S. lavendulae* and the actinorubin and lavendulin strains of Kelner and Morton (119).

ANTIBIOTIC 136. Crystalline derivatives of this antibiotic have not been obtained. The titration curve indicates that both strong and weak basic groups are present. The hydrochloride salt is soluble in methanol and readily precipitated by acetone. The maximum thermostability was shown in aqueous solution at pH 2.0, wherein only 10% of the activity was lost after heating one hour at 100°. Under similar conditions at pH 8.0, 90% of the activity was lost. Like streptothricin, antibiotic 136 is highly active against Gram-positive, Gram-negative, acid-fast organisms and higher fungi. The ratio of the activity of antibiotic 136 in broth to its activity in agar is 75 times as great as the ratios for streptomycin or streptothricin. The LD_{50} of the antibiotic is 0.2 mg./18- to 24-gram mouse, given either subcutaneously or intravenously. Death does not occur until three to five days after injection. Ten times the dose which is toxic parenterally has no ill effects when given by mouth.

Streptomyces lavendulae 136-B, which produces antibiotic 136, also produces streptothricin. However, Bohonos et al. (19) also

obtained antibiotic 136, or preparations very similar to it, from 11 other strains of *Streptomyces*. Some of these were similar to the 136-B strain, and the remainder were cultures of a white-spored form of *Streptomyces* sp.

ANTIBIOTIC 3510 (Grisein-like factor). According to Garson and Waksman (75), antibiotic 3510 has not been purified beyond a crude powder assaying 12,000 to 20,000 *Escherichia coli* units per gram. It is soluble in water and insoluble in organic solvents, and is active against both Gram-positive and Gram-negative bacteria, especially those of enteric origin. Its narrow bacterial spectrum is less than that shown by grisein. Resistance is readily developed by organisms sensitive to the antibiotic, but, unlike grisein, it is selective in action instead of being general. It is relatively stable to heat, is not inhibited by serum and shows protective action in vivo against Gram-negative pathogens injected into mice. A second factor which inhibits the growth of mycobacteria could not be isolated from the metabolic solutions containing the grisein-like antibiotic. It is produced by *Streptomyces griseus*, Waksman's No. 3510, and by *S. griseus* NRRL B-1248 (13*a*).

ANTIMYCIN A ($C_{28}H_{40}O_9N_2$) (Dunshee et al., 55). A colorless crystalline nitrogenous phenol which is weakly acidic, m.p. 139–140°, optically active, $[\alpha]_D^{25} + 64.8°$ (C = 10 in chloroform). Studies by Bumpus et al. (34) on the saponification of antimycin A yielded a neutral compound which, upon further mild alkaline hydrolysis, gave a compound, $C_{11}H_{20}O_3$. The aqueous phase, adjusted to pH 3, yields a nitrogenous phenol, $C_{11}H_{20}O_5N_2$, and L(+) methylethylacetic acid, $C_5H_{10}O_2$. According to Tener (208), crystalline antimycin A may be separated into three biologically active components (two major and one minor) which appear to be similar, perhaps differing only in the fatty acid component. Schneider et al. (189*a*) chromatographed antimycin broths and found that the fraction corresponding to antimycin A, accounting for 85–90% of the total activity, is fast moving, whereas a second portion, " antimycin B ", with 10–15% of the activity, is nearly stationary. The antimycin B fraction may well consist of two or more unresolved components. The parent compound is freely soluble in ether, ethyl alcohol, acetone and chloroform; very slightly soluble in petroleum ether, benzene and carbon tetrachloride; and insoluble in water and 5%

aqueous solutions of hydrochloric acid, sodium carbonate and sodium bicarbonate. Alcoholic solutions appear to be stable indefinitely. No sensitivity to light or atmospheric oxygen has been noted. It is an extremely potent fungicide, producing an inhibitory effect against *Nigrospora sphaerica* at dilutions as high as 1:800,000,000. Leben and Keitt (137, 138), who originally investigated antimycin A, tested its growth-inhibiting properties on 33 higher fungi, all of which were affected. Kido and Spyhalski (120*a*) found that antimycin A is very effective against certain insects, showing specific killing properties when ingested by various insects of the order Coleoptera, but had no effect on test insects of the order Lepidoptera. It was three to four times as effective as di (*p*-chlorophenyl) methyl carbinol against the red spider mite, *Tetranychus* sp. The designation, antimycin A, refers only to the crystalline compound, since there are indications that other factors in addition to antimycin A are present in crude ethanol extracts. The strain of *Streptomyces* producing the complex has not yet been identified.

ANTI-PHLEI FACTOR. Studies by Ouchi (164*a*) have shown that this factor is a basic substance whose crystalline helianthate decomposes at 243–247°. The hydrochloride is a white amorphous powder which gave negative tests for maltol, Sakaguchi, Vole's sulfur, biuret reaction, Millon and Molisch. It is soluble in methanol and water, and insoluble in acetone and ether. The substance is most stable to heat (100° C. for 60 minutes) at pH 5.0 and considerably less stable to heating on the alkaline side. It is most active against *Mycobacterium phlei,* somewhat less against *M. avium* and *M. tuberculosis,* and weakly active against *M. smegmatis.* Slight activity was exerted against *B. subtilis* and *B. anthracis* and none against Gram-negative bacteria. Injections of 10 and 20 mg. of crude substance (25,000 dilution units/mg.), either intramuscularly or intravenously into 16–17-gram mice, were non-toxic. The strain producing the anti-phlei factor, No. 364, was identified as *Streptomyces aureus* (164*a*).

As pointed out by Ouchi, the above factor shows some resemblance to the anti-smegmatis factor of Kelner and Morton (118). A comparison of the anti-phlei factors of Kurosawa (128) and Ouchi could not be made by the reviewer because detailed information on the former has not appeared in the literature.

ANTI-SMEGMATIS FACTOR. Chemical purification of this factor was not attempted by Kelner and Morton (118). When crude filtrates were tested against 60 species of microorganisms, including 20 genera of bacteria, they inhibited the growth of *Mycobacterium smegmatis, M. phlei* and *M. tuberculosis* var. *hominis* (strain 607). *M. tuberculosis* var. *bovis* was not affected. Crude filtrates withstood 30 minutes heating at 100°, and two ml. injected intraperitoneally into 17- to 20-gram white mice had no apparent toxic effect. *Streptomyces* A-82, which produces the antibiotic, resembles *S. lavendulae* but is not identical with it.

ASCOSIN. Studies on this antibiotic by Hickey et al. (*97a*) indicate that purified preparations vary from brown to yellowish-orange in color. Ascosin appears to be related to fungicidin in certain respects but the two agents are different on the basis of their ultraviolet absorption curves.

Ascosin is very soluble in pyridine, quinoline, pyrrole and certain other amines, particularly when mixed with water, and is relatively insoluble in acetone, ether, benzene, chloroform and petroleum ether. It is rapidly inactivated by strong bases or strong acids, and the optimum pH range for stability lies between 5 and 7.5. It is quite effective in inhibiting the growth of various yeasts and shows strong activity against some filamentous fungi but very low activity against others. The oral toxicity of ascosin in mice is low, i.e., greater than 500 mg./kg., and the LD_{50} acute toxicities in mice, given subcutaneously and intravenously, were 168 mg./kg. and 12.5 mg./kg., respectively. The ascosin-producing strain has been assigned the name *Streptomyces canescus* (*97a*).

AUREOMYCIN ($C_{22}H_{23}O_8N_2Cl$), crystalline free base; Pepinsky and Watanabe (*169a*), a correction of the formula previously prepared by Dunitz and Leonard (*53b*). This antibiotic was discovered by Duggar and co-workers of the Lederle Laboratories (53). Studies on the alkaline degradation products of both aureomycin and terramycin were made by Kuhn and Dury (*126a*). Dunitz and Robertson (54) conducted various X-ray and optical measurements on the hydrochlorides of aureomycin and terramycin, and concluded that the two molecules must be very similar in shape and orientation. Later, various studies on the degradation products of aureomycin by Waller et al. (*242a, b, c*) and Hutchings et al. (106)

served to elucidate a structural formula for aureomycin which differed from that of terramycin in the position of the chlorine atom of aureomycin on C_{10} as numbered by the Lederle group and in the absence of a hydroxyl group on C_{12}. Stephens et al. (195*b*) confirmed these findings and suggested the name " tetracycline " for the major portion of the structure which is common to both aureomycin and terramycin. The structural formula of aureomycin is not shown in Table IV, since Waller et al. (242*c*) indicated two possible positions for the attachment of the dimethyl amine group on the ring containing the carboxamide group. According to Broschard et al. (31), the yellow crystalline free base has the following properties: m.p. 168–169° (corrected), $[\alpha]_D^{23}$ −275.0° (methanol), soluble in water, 0.5–0.6 mg./ml. at 25°, very soluble in Cellosolves, dioxane, and Carbitol; slightly soluble in methanol, ethanol, butanol, acetone, ethyl acetate, and benzene; insoluble in ether and petroleum ether. The crystalline hydrochloride is considerably more soluble in water than the free base.

The antibiotic is most stable at pH 2.0; at higher pH's the temperature of incubation has a marked effect on stability. Dornbush and Pelcak (52*b*) held aureomycin solutions at pH 1.0 through 10 at 4° ± for approximately 18 hours with no loss in activity, whereas at pH 7.0 more than half of the activity was lost after overnight incubation at 37°.

The antibiotic exhibits a very wide spectrum, inhibiting the growth of Gram-positive and Gram-negative bacteria, rickettsiae and certain of the so-called larger viruses, and has given promise in the treatment of amebiasis in humans (McVay et al., 151). Bryer et al. (32) reported mice survived 50 mg./kg., intravenously, but not 100 mg./kg. The same animals survived up to 3000 mg./kg. by the subcutaneous route. Repeated subcutaneous and intramuscular doses of aureomycin were well tolerated in rats and dogs except for local irritation, weight loss and anorexia. The antibiotic is produced by selected strains of *Streptomyces aureofaciens* (Duggar, 53*a*). One such representative culture is Lederle A-377. Because of its low toxicity and its wide range of effectiveness, aureomycin is currently produced in large quantities and finds wide use in medical practice and in feed supplementation.

AUREOTHRICIN ($C_9H_{10}N_2O_2S_2$, revised structural formula by Celmer et al. (43*a*)). See also Thiolutin. The first reports of this

compound were those of Umezawa et al. (218, 220) who crystallized the antibiotic and assigned to it the designation "aureothricin". Subsequently Maeda (145) determined its empirical formula and studied its chemical properties. The golden yellow needles have no definite melting point and decompose at 256–257°. Like thiolutin, aureothricin is only slightly soluble in organic solvents and insoluble in water. It is very stable to heat and to strong acid. Although activity against higher fungi was not given, it is effective against various Gram-negative and Gram-positive bacteria. Mice survived subcutaneous injections of five mg. suspended in gum acacia, but ten mg. by the same route was fatal. The aureothricin-producing strain (218) resembled *Streptomyces lipmanii* and *Actinomyces farcinicus* (*Nocardia farcinica*) but could not be definitely identified. Aureothricin and thiolutin are closely related compounds with similar biological and chemical properties.

BORRELIDIN ($C_{28}H_{43}NO_6$). A crystalline acid (Jampolsky and Goldberg, 111) with m.p. 145–146°, $[\alpha]_D^{27} = -28°$ in ethanol. Its ultraviolet absorption spectrum in isopropanol shows a maximum at 256 mμ ($E_{1\,cm.}^{1\%} = 550$), indicating a site of conjugated unsaturation in the molecule. It is soluble in various organic solvents, such as butyl acetate, ether and benzene; the extent of solubility in water is not given. The antibiotic is active against *Sarcina lutea* and certain micrococci, and is effective against several strains of *Borrelia* in vivo (Berger et al., 15). Borrelidin was found by Grunberg et al. (83) to enhance the activity of penicillin in certain experimental infections in mice; and, interestingly enough, concentrates of crude penicillin have been reported to contain enhancement factors which exhibit some properties of borrelidin. Toxicity studies by Buck et al. (33) showed that borrelidin is a comparatively toxic compound. The LD_{50} subcutaneous dose for mice is 74.7 mg./kg. and for rats, 1.78 mg./kg. The more resistant mice and chicks could not tolerate more than 1.5 mg./kg. over periods of 15 to 21 days. The borrelidin-producing strain has been assigned the name *Streptomyces rochei* (15).

CACAOMYCETIN. Wakaki et al. (228) have recently reported the isolation of a yellow sirup (10,000 *Aspergillus niger* units/mg.) which, when submitted to alumina chromatography and paper partition studies, provided evidence of four distinct antibiotics. The

complex was slightly soluble in water and soluble in many organic solvents, including carbon tetrachloride. It was stable in neutral and weak acid solutions but rapidly inactivated in alkali. High activity was exerted against saprophytic phycomycetes and ascomycetes, but little or no activity was shown against bacteria. The MLD in mice by subcutaneous injection of peanut oil suspensions was 300 mg./kg. The cacaomycetin strain closely resembles *Streptomyces cacaoi* (228).

CARDICIN. Studies by Machlowitz et al. (144*a*) have shown that highly purified cardicin is a white acidic compound. The acid form is insoluble in water, saline solution, ether and acetone but soluble in methanol, ethanol and large volumes of hydrous butanol. The sodium salt is insoluble in ether and cold anhydrous butanol and soluble in water, methanol, ethanol and large volumes of hydrous butanol. The compound is relatively stable over a wide range of temperature and pH and is indefinitely stable at 5°.

A preparation showing 1340 *B. subtilis* units/mg. also showed selective activity against *M. pyogenes* var. *aureus, Mycobacterium* 607 and certain higher fungi. Gram-negative bacteria were unaffected at 200 *B. subtilis* units/ml. Staphylococcal, streptococcal and *E. coli* bacteriophages were inhibited at 100 units, and cardicin showed antiviral activity against influenza virus strains PR-8, Lee and FM-1. In six different samples of cardicin, representing various degrees of purification, a constant ratio of *B. subtilis* unitage to antiviral unitage was observed, suggesting that a single entity was responsible for both activities.

Cardicin is quite toxic to mice. When injected either subcutaneously or intraperitoneally into mice, 125 *B. subtilis* units (approx. 0.1 mg.) was fatal within 48 hours. Extensive liver damage was noted upon autopsy. Cardicin is obtained from cultures of three species of the genus *Nocardia,* two of which also produced streptothricin-like antibiotics (144*a*).

CATENULIN. Davisson et al. (52*a*) have recently described this new member of the neomycin group. Crude catenulin could be further purified by crystallization of the helianthate or of the *p*(*p*^{1}-hydroxy-phenylazo)-benzene sulfonate salt from hot water, and was optically active, $[\alpha]_D^{25} = +33°$ (C, 1% methanol). The sulfate was prepared from the dye salt and, after drying at 100° in vacuo,

analyzed as C, 31.53, 31.38; H, 6.07, 6.23; N, 7.92, 7.93; and SO_4, 28.11 and 28.13; optically active, $[\alpha]_D^{25} = +51.9°$ (C, 1% water). The ultraviolet spectrum shows only end absorption, and the infra red absorption is typical of a polypeptide spectrum.

Catenulin is similar to neomycin B, and the sulfate analysis closely resembles that obtained for neomycin B, although the rotation is lower. However, catenulin may be distinguished from neomycins A and B, chromatographically, and is stable in aqueous solution (0.25 mg./ml.) for 30 days at a range from pH 1.5–10.0, while neomycins B and C are reported to be unstable in acid solutions. The antibiotic is active against several strains of mycobacteria, including the streptomycin-sensitive and -resistant strains of *M. tuberculosis*, H37RV. The acute intravenous LD_{50} dose for mice is 125 mg./kg. Daily injections of 100 mg./kg. in cats produced definite indications of neurotoxicity and cumulative toxicity, and resulted in a significant number of deaths within one month. The catenulin-producing strain, which is to be described later, is not *S. fradiae*.

CHLOROMYCETIN * (chloramphenicol), $C_{11}H_{12}N_2O_5Cl_2$, D-*threo*-N-(1,1¹-dihydroxy-1-*p* nitrophenylisopropyl)-dichloroacetamide (Rebstock et al., 176) was first reported by Ehrlich et al. (61) and independently investigated by Carter et al. (39) and Gottlieb et al. (77). See structural formula in Table IV. Studies on the properties of this antibiotic by Bartz (7) showed that it crystallizes from water or ethylene dichloride as colorless needles or elongated plates with m.p. 149.7–150.7° (corrected). It is optically active, $[\alpha]_D^{25} = -25.5°$ (ethyl acetate), and has a very characteristic absorption spectrum in the ultraviolet, $E_{1\text{ cm.}}^{1\%}$ 298 and 278 mμ. Chloromycetin is very soluble in methanol, ethanol, butanol-1, ethyl acetate and acetone; fairly soluble in diethyl ether; and insoluble in benzene and petroleum ether. The solubility in water, 70% aqueous propylene glycol and propylene glycol (25°) is about 2.5, 60.6 and 150.8 mg./ml., respectively. The antibiotic is remarkably stable in distilled water; no loss of activity was noted on boiling at 100° for five hours. Chloromycetin has the distinction of being the only medically useful antibiotic which can be synthesized on a practical scale (Controulis et al., 47). The antibacterial spectrum is similar to that of aureomycin and terramycin, i.e., affecting

* Trademark name.

Gram-positive and Gram-negative bacteria and rickettsiae. The LD_{50} intravenous dose in propylene glycol for 20-gram white mice is 245 mg./kg. One gram/kg., orally, as an acacia suspension produced temporary depression in mice, with recovery in less than 24 hours (Smith et al., 195). Reports of a number of fatal cases of aplastic anemia, following chloramphenicol therapy, resulted in a ruling by the Food and Drug Administration that the labelling on the drug be revised to caution physicians against indiscriminate use of it.

Chloromycetin-producing strains (isolated in this country) have been named *Streptomyces venezuelae* (Ehrlich et al., 60). Umezawa et al. (219) isolated a chloromycetin-producing strain, No. 102, which differed from *S. venezuelae* and to which was assigned the name *Streptomyces omiyaensis*.

The antibiotic was the first drug to supplant streptomycin in the therapy of many diseases caused by Gram-negative organisms and is produced in large quantities, both synthetically and biologically. It is especially useful in treating typhoid fever and rickettsial diseases.

CHROMIN. (See Fungicidin). Wakaki (227*a*) has isolated this fungicidin-like factor in the form of small white needles. It gives a negative test for ninhydrin and positive Tollens and Sakaguchi tests. The ultraviolet absorption spectrum is identical with that of fungicidin RAW. Crystalline chromin is insoluble or sparingly soluble in organic solvents and water, but is soluble in alkaline water. It is stable at pH 7.0 but unstable in acids or alkali. Fungicidin RAW inhibits the growth of *Aspergillus niger* at 330 μg./ml. (227*a*), whereas only 0.8–1.0 μg./ml. of chromin is required to prevent growth of the same culture. The chromin-forming strain, C-6, was thought to be a new species of *Streptomyces* exhibiting chromogenic properties (227*a*). A description of the strain has been published by Wakaki et al. (228*a*).

CINNAMYCIN. This factor is a polypeptide antibiotic which was studied by Benedict et al. (11). Paper chromatography of acid and alkaline hydrolysates of cinnamycin revealed eight major ninhydrin-reacting spots, tentatively identified as lanthionine, aspartic acid, arginine, glutamic acid, proline, phenylalanine, valine and the $C_7H_{14}N_2O_4S$ amino acid that Alderton, as cited by Lewis and Snell (142*b*), found in subtilin. The C_7 acid in subtilin has been identi-

fied by Alderton as a β-methyl lanthionine (Lewis, 142*a*). Aqueous solutions of cinnamycin are optically active, and end absorption is shown in the ultraviolet with a shoulder at 250–260 mμ. It is soluble in water, 50% aqueous ethanol, 80% methanol, butanol and glacial acetic acid. Quantitative tests on the hydrochloride and picrate showed the presence of nitrogen and sulfur, and the absence of halogen in the free base. The hydrochloride gave positive Sakaguchi and biuret tests and negative tests for ninhydrin, ferric chloride, Molisch, maltol, Tollen and reducing sugars. It is stable to 30 minutes heating at 94° within a pH range of 2.0 to 5.4, but unstable in alkaline solution. It is not inactivated by light or by the enzymes pepsin or trypsin. It is effective against certain Gram-positive rods and acid-fast bacteria, and inactive against Gram-positive cocci. A second unidentified antibiotic is produced in culture along with cinnamycin by *Streptomyces cinnamoneus* which we are currently describing as a new species (11*a*).

COELICOLORIN. A purplish-red powder, m.p. 142–146°, which was studied by Hatsuta (92) who found that it was red at pH 5, purple at pH 6–7, and green at pH 8.0 or above. Below pH 5 it was insoluble in water, but slightly soluble at pH 6–7 and highly soluble at pH 8.0 or above. It is soluble in acetone, ethyl acetate, chloroform, benzene, ethanol, methanol and ether; insoluble in petroleum ether. It is active primarily against Gram-positive organisms and requires a concentration of about 100 μg./ml. to inhibit the growth of various Gram-negative pathogens. When 20-gram mice were injected intraperitoneally with coelicolorin in olive oil suspensions, three out of four animals survived a dose of five mg.; the LD_{50} dose was ten mg. per mouse. The antibiotic was named after *Streptomyces* A-134, identified as *S. coelicolor*.

DEXTROMYCIN. As reported by Ogata (162), dextromycin is an organic base which may be recovered from culture by methods applicable to streptomycin or streptothricin. It does not yield maltol on alkaline hydrolysis, is Sakaguchi negative and diffuses slowly through agar. The specific rotation of the hydrochloride is $[\alpha]_D^{25}$ +61.0; m.p. of the helianthate, 227–228°. Although no approximate molecular weight figure is available, certain chemical reactions of the substance suggest that it is more closely related to neomycin than to streptomycin or streptothricin.

It inhibits the growth of Gram-positive, Gram-negative and acid-

fast microorganisms in the same range as does streptomycin and is stable to 30 minutes heating at 100°. The LD_{50} toxicity for mice is 50 mg./kg., intravenously, and 750 to 1000 mg./kg., subcutaneously. It is produced by *Actinomyces* A-1404 (162) which was not identified as to species. According to Maeda (145*a*), the description of this strain, in the opinion of Umezawa, closely resembles that of *Streptomyces fradiae*.

EHRLICHIN. Crude concentrates of ehrlichin were reported by Groupé et al. (82) to be heat stable (100° for 40 minutes), resisted tryptic digestion and were non-dialyzable. This agent has specific antiviral activity, inhibiting influenza B virus (Lee), influenza A virus (PR-8) and infectious bronchitis virus (IBV) by the contact test. Activity in vivo is limited to influenza B virus (82*a*). No activity was shown against common Gram-positive and Gram-negative forms. The *Streptomyces* sp. which produces this factor is reported to be a non-streptothricin-producing strain of *S. lavendulae*.

ENDOMYCIN. Studies conducted by Gottlieb et al. (78) show that endomycin is a high molecular weight acid containing approximately 3.7% nitrogen. The acid form of endomycin is soluble in alcohols, methyl cellosolve, and sparingly soluble in dioxane. It is insoluble in water, ether, chloroform, benzene, ethyl acetate and other fat solvents. It is stable to 100° for at least 30 minutes and to autoclaving at 15 lb. for 20 minutes. No inactivation occurred during a 24-hour period at a pH range of 1.1 to 11.5. A major portion of this factor, as suggested by its name, is found in the mycelium. It is active against many higher fungi, Gram-positive bacteria and some Gram-negative bacteria. Mice withstood injections of 500 mg./kg. but succumbed within 48 hours to injections of 1000 mg./kg. *Streptomyces* 9–20, which produces endomycin, has been named *Streptomyces endus* (3*a*). Two other antibiotic factors are produced concurrently with endomycin—antibiotic 9–20F–1 which is ether-soluble and appears to have a spectrum similar to that of endomycin, and antibiotic 9–20B which has no activity against higher fungi.

FLAVACID. This factor was first described by Takahashi (205*d*) as a yellowish crystalline powder which melts with decomposition at 102–105°. It is weakly acidic and can be precipitated as the barium, calcium, lead, zinc or procaine crystalline salts. Color tests with ninhydrin or ferric chloride were negative. The sodium salt

is soluble in methanol and hydrous acetone; relatively soluble in water and ethanol; less soluble in acetone and butanol; and insoluble in ether, ethyl acetate, chloroform and benzene. A neutral solution in butanol, acetone and water showed a yellowish tinge which changed to green upon acidification or to red with addition of alkali. In both instances there was a loss of activity. The antibiotic shows good activity against certain Gram-positive bacteria but seems to be most active against various yeasts and somewhat less effective against some other higher fungi. Intraperitoneal injections in mice showed an LD_{50} of 50 mg./kg. The antibiotic was effective in treating human cases of trichophytosis and *Trichomonas vaginalis*. The flavacid-producing strain, O-2, closely resembles *Streptomyces flavus* (205*d*). A second factor produced by the same strain was called " D-substance ". This antibiotic crystallized as white needles (m.p. 124–125°), and its low antifungal activity and high toxicity did not warrant further investigation.

FLAVOMYCIN. This basic antibiotic is reported by Arai (4) to be a light yellow powder in the form of the hydrochloride, and can be obtained as a crystalline reineckate which decomposes at 210–212°. The limited information available on its chemical and physical properties suggests that it is very similar to neomycin. Aiso et al. (1) found flavomycin to be thermostable and effective against Gram-positive, Gram-negative and acid-fast organisms. The MLD against mice was 200 mg./kg. intravenously, and 500 mg./kg. subcutaneously. It is produced by *Streptomyces* No. 320 which resembles *S. microflavus, S. fradiae* and *S. roseochromogenus* without being identical to any of the three. It has been named *Streptomyces roseoflavus* (4). In Umezawa's opinion (Maeda, 145*a*), this strain is closely related to or possibly identical with *S. fradiae*.

FRADICIN. This antibiotic has been assigned a tentative empirical formula, $C_{30}H_{34}N_4O_4$, and was shown by Hickey and Hidy (97) to be a weak base, forming a hydrochloride obtained as needles. Two relatively simple methods for the isolation of crystalline fradicin from fermentation media were devised by Hidy and Hickey (98). The most highly purified crystalline products are light greenish-yellow, showing no definite melting point, but they darken without melting at 180 to 300°. It is soluble in dioxane, in ethylene dichloride and related chlorinated hydrocarbon solvents,

and in propylene glycol; practically insoluble in petroleum ether, cyclohexane, xylene, water, methanol and ethanol, i.e., less than 0.05 mg./ml.; and optically active, $[\alpha]_D^{25} + 65°$ (c 1.0, 1,4-dioxane). Alkali fusion yields a volatile product which gives positive pine splint and Ehrlich tests for a pyrrol. As originally indicated by Swart et al. (203), fradicin, formerly designated as "factor X", is a highly active antifungal agent, inhibiting the growth of yeasts and many pathogenic fungi. Bacteria are practically unaffected. Purified fradicin was stable for 30 minutes at 100°, pH 7.0. The LD_{50} dose of crystalline fradicin for mice, given orally or intraperitoneally, is four mg./kg. When employed as a hydrophilic ointment in skin tests on rabbits, it showed considerable irritation at 500 μg./gm. The antibiotic is produced by the neomycin-forming strain of *Streptomyces fradiae* (205).

FUNGICIDIN. This factor was first reported by Hazen and Brown (93). The partially purified product was a yellow powder containing 1.5% Kjeldahl nitrogen, no sulphur or halogens, and gave negative tests for carbohydrate or protein. It is sparingly soluble in methanol and ethanol and relatively insoluble in most organic solvents and water. It is very unstable in aqueous solutions at pH 2.0 and 9.0. The antibiotic, which is formed in the mycelium on surface culture, shows strong reducing properties and is believed to be a relatively small molecule. A wide variety of saprophytic and pathogenic fungi were inhibited in a range from 1.56 to 6.25 μg./ml., whereas bacteria were not affected by 100 μg./ml. The LD_{50} for mice, intraperitoneally, was 20–26 mg./kg. Subcutaneous administration permitted doses of two gm./kg. without killing the animals. In vivo tests indicated that fungicidin has therapeutic value in histoplasmosis and cryptococcosis induced in mice. *Streptomyces* 48240 produces, in addition to fungicidin, an antibiotic which is secreted into the medium and which resembles Acti-dione. The responsible culture was not identified as to species.

Raubitscheck et al. (175*a*) recently described an antifungal antibiotic isolated from shaken cultures employing a strain of *Streptomyces aureus,* Waksman's No. 3569. The ultraviolet absorption spectrum of this factor and that of fungicidin appeared to be identical. Further studies were made on the antibiotic from Waksman strain 3569 by Schwartz et al. (191*a*). This product, for which

Waksman (229*c*) suggests the designation "fungicidin RAW", was supplied as preparation C 381. It was found to be very effective against *Coccidioides immitis* infections in mice and considerably less toxic than fradicin. When crude C 381 was tested for acute toxicity in mice (191*a*), the LD_{50} dose was 204 mg./kg. intraperitoneally, and 532 mg./kg. when injected subcutaneously.

GRISEIN ($C_{40}H_{61}N_{10}O_{20}SFe$). Grisein is a weak acid, obtained as a red amorphous powder which, thus far, is the only highly purified actinomycete antibiotic shown to contain iron in the molecule. Kuehl et al. (126) found that pure grisein is soluble in water and phenol, and insoluble in all common organic solvents. It has characteristic absorption spectra with maxima at 2650 Å, $E^{1\%}_{1\text{ cm.}}$ 108, and 4200 Å, $E^{1\%}_{1\text{ cm.}}$ 28.9. Hydrolysis of pure grisein indicated that two amino acids, glutamic and a second, unidentified, are probably component parts of the molecule. These investigators also recovered 3-methyluracil from grisein. The ferric iron from pure grisein (300,000 *Escherichia coli* units/mg.) could be removed by 8-hydroxyquinoline which gave a colorless iron-free grisein assaying 120,000 units/mg. The stoichiometric amount of ferric chloride may be reintroduced into the molecule, resulting in restoration of the higher level of antibiotic activity.

The crude grisein preparations originally studied by Reynolds et al. (181) were stable to heat (ten minutes at 100°) and showed very narrow selective inhibition against Gram-positive and Gram-negative bacteria. Susceptible strains readily acquired resistance to grisein. Toxicity tests reported by Reynolds and Waksman (182) showed that the antibiotic is well tolerated by experimental animals, even in concentrations of 500,000 units/kg. This material probably did not contain more than 1800 *E. coli* units/mg. Grisein is produced by certain strains of *Streptomyces griseus*. One such representative strain is Waksman's *S. griseus* No. 3478.

GRISEOLUTEIN. Calculations from the elemental analysis given by Umezawa et al. (216) for griseolutein suggest the empirical formula $(C_{8.6}H_{7.6}NO_3)_n$ (no approximate molecular weight given). Qualitative tests indicate no halogen or sulfur. The antibiotic is an acidic substance which can be recrystallized from ethyl acetate as yellow needles. It decomposes at 193°. It is insoluble in water, ether and benzene, slightly soluble in ethyl acetate and alcohol. It

is soluble in a sodium bicarbonate solution but is unstable in alkali and more stable at pH 2.0 than at neutrality. Griseolutein is appreciably more active against Gram-positive than against Gram-negative bacteria. Ten mg. of antibiotic injected subcutaneously into ten-gram mice did not induce toxic reactions. The name *Streptomyces griseoluteus* was assigned by Umezawa and co-workers to strain P-37 which produces the factor.

HELIXIN. (See appended list).

HYDROXYSTREPTOMYCIN ($C_{21}H_{39}N_7O_{13}$). In 1949, Hosoya et al. (102) first reported on a streptomycin-like compound called "reticulin" which was produced by a strain identified as *S. reticuli*. The crystalline reineckate decomposed at 166–169°, compared with streptomycin reineckate at 162–164° (corrected) Fried and Wintersteiner (73) and 160–163° (corrected) by Vander Brook et al. (224). The structure of reticulin was not determined in the earlier work, but its resemblance to streptomycin was emphasized (102) in that a streptomycin-resistant strain of *Escherichia coli* was also resistant to reticulin and a reticulin-resistant *E. coli* strain was not inhibited by 3200 μg./ml. of streptomycin. In 1950 the first published report on hydroxystreptomycin was that of Benedict et al. (13) of the Northern Regional Research Laboratory, and studies on the chemistry of the factor were made by Stodola et al. (196) of the same Laboratory. The same antibiotic was independently investigated by Grundy et al. (84) of the Abbott Laboratories. This antibiotic differs from streptomycin only in having a hydroxymethyl group instead of a methyl group in the streptose portion of the molecule (see Table V). Work was continued on the nature of reticulin, and Uno (223*a*) found that highly purified reticulin hydrochloride gave both pyromeconic and isokojic acids upon alkaline hydrolysis. He indicated that reticulin and hydroxystreptomycin in admixture could be separated by paper strip chromatography when resolved in 80% phenol–5% piperidine or butanol : water : acetic acid (4 : 2 : 1). However, Komatsu (122*a*) stated that confirmation of these results with the same solvents could not be obtained in Hosoya's laboratory, and Hosoya et al. (105*d*) presented evidence that the two antibiotics are identical. They have not been able to account for the presence of pyromeconic acid which they found upon alkaline hydrolysis of both reticulin and hydroxy-

streptomycin. Since the Japanese workers had not chemically identified reticulin and the name hydroxystreptomycin readily characterizes our product and that of Grundy et al. (84), the reviewer chose to group these antibiotics together under the name of the product which was first chemically identified. Hydroxystreptomycin is an organic base, very soluble in water, less soluble in lower alcohols, and insoluble in other organic solvents. Hydroxystreptomycin trihydrochloride showed a specific rotation in water of $-91°$. Like streptomycin, it is stable in weak acid and weak-base solutions at room temperature. Its bacterial spectrum shows activity against Gram-positive, Gram-negative and acid-fast organisms. Grundy et al. (85) conducted acute, chronic and eighth cranial nerve toxicity tests with both hydroxystreptomycin and dihydroxystreptomycin on mice and rabbits. The results of these studies indicate that the toxic properties of the two antibiotics are essentially the same as those of streptomycin and dihydrostreptomycin, respectively. Ambrose (2) found the LD_{50} dose of hydroxystreptomycin trihydrochloride and streptomycin sulfate in white mice, subcutaneously, to be 865 mg./kg. and 970 mg./kg., respectively. The antibiotic is produced by a variant of *S. rubrireticuli* (105*d*), formerly referred to as *S. reticuli* (102); *Streptomyces griseocarneus,* NRRL B-1068 (Benedict et al., 12), and by *Streptomyces sp.* NA 232-M1 of Abbott Laboratories (85). This latter culture appears to be a strain of *S. griseocarneus,* although it shows some minor physiological differences.

ILOTYCIN * (Erythromycin, Lilly), $C_{34\text{-}36}H_{60\text{-}65}NO_{11\text{-}14}$ molecular weight approximately 725. Ilotycin, as reported by McGuire et al. (150*a*), is a white crystalline base which readily forms salts with acids. Electrometric titration in 66% dimethylformamide-water, and in water in the pH range 2–12 shows one titratable group with a pK_a^1 of 8.8. The optical rotation, $[\alpha]_D^{25}$, is $-78°$ at a concentration of 1.99% in alcohol when the product is dried in vacuo for four hours at room temperature. The ultraviolet absorption spectrum is characterized by a single broad peak of weak intensity having a maximum at 280 mμ at pH 6.3 with E_{mol}, the molar extinction, about 50. It is extremely soluble in acetone, chloroform, acetonitrile, ethyl acetate and various alcohols, and

* Trademark name.

moderately soluble in ether, ethylene dichloride and amyl acetate. It is soluble in water to the extent of about two mg./ml.

The compound is highly active against various Gram-positive and acid-fast bacteria and is effective against some pathogens of the *Brucella, Hemophilus* and *Neisseria* group. As shown by Powell et al. (171*c*), more than 50 μg. per ml. is required to inhibit the growth of certain strains of *Aerobacter, Escherichia, Salmonella* and *Shigella*. The bacterial spectrum constitutes, therefore, a resemblance to that of penicillin and a dissemblance to terramycin, aureomycin and Chloromycetin. The growth of erythromycin-resistant and corresponding non-resistant parent strains of *M. pyogenes* var. *aureus* are equally inhibited by other commonly used antibiotics. This indicates that there is no tendency for development of cross-resistance. Staphylococci resistant to other antibiotics are susceptible to Ilotycin, in vitro, and preliminary tests show some activity against *M. tuberculosis* H37RV in vivo and in vitro. Viruses of the lymphogranuloma venereum and mouse meningopneumonitis group and various rickettsiae are susceptible to Ilotycin. Both in vivo and in vitro activity are reported against *Endamoeba histolytica, Spirocheta novyi, Trichomonas vaginalis* and oxyurids (pinworms) in mice. The toxicity data on "Ilotycin" and very favorable. Mice tolerate single oral doses of 2000 mg./kg., and doses of 3000–4000 mg./kg. resulted in an occasional death among these test animals. The LD_{50} dose for mice subcutaneously is 1800 mg./kg. Low acute and chronic toxicities are also reported in dogs. The antibiotic has shown encouraging results in the treatment of human cases of hemolytic streptococcus infections and pneumococcus infections. The "Ilotycin" production strain has been identified as *Streptomyces erythreus* (150*a*).

LAVENDULIN. (See Actinorubin). This antibiotic was purified by Junowicz-Kocholaty and Kocholaty (116) as a crystalline helianthate ($C_{49}H_{63}O_{18}N_{13}S_3$). The empirical formula calculated for the free base from the above helianthate was unsatisfactory. The salt formed when lavendulin is reacted with sodium helianthate is quite soluble in 80% methanol and almost insoluble in 20% methanol. The free base is said to be thermostable in neutral solution. Lavendulin inhibits Gram-positive, Gram-negative and acid-fast bacteria. Intraperitoneal injection of 25 μg. (0.078 μg. equals a dilution unit against *E. coli*) protected mice against *Klebsiella*

pneumoniae. The LD_{100} dose, intraperitoneally, for mice was 0.5 mg. (158). It is produced by *Streptomyces* A-10 (120) which resembles *S. lavendulae* in certain respects.

LITMOCIDIN. As suggested by the name, litmocidin is an antibiotic pigment which is red under acid conditions, violet at neutrality and blue in alkali (Brazhnikova, 20); melting point 144–146° (decomp.). It is soluble in ethanol, acetone, ether and amyl acetate under acid conditions. The red form is slightly soluble in water, the blue (alkaline) form readily so. It is stable to boiling in the acid form, but the alkaline form is rapidly destroyed. Both forms inhibit the growth of *Staphylococcus aureus* at a dilution of 1:4,000,000. According to Gause (76), it also strongly inhibits the growth of streptococci, *M. tuberculosis* and *Vibrio comma,* but exerts only a weak effect on most Gram-negative forms. Intraperitoneal injections of 3000 units caused no harm in mice, whereas 10,000-unit doses were invariably fatal. The designation *Proactinomyces cyaneus-antibioticus* has been assigned to the antibiotic-producing culture (76).

LUTEOMYCIN. The crystalline hydrochloride form of this antibiotic, as reported by Hata et al. (90, 91), appears as yellowish-orange rhomboid crystals which change from yellow to brown at 160° and finally become black. It is decomposed at 199°. A positive quinone reaction is obtained with sodium carbonate and hydrogen peroxide, and an aqueous solution of the antibiotic is said to be yellowish-orange in acid, slightly brown at neutrality and reddish-purple at pH 7.8. The maximum absorption band is at 430–420 $m\mu$ with .1 *N* HCl. It is readily soluble in chloroform and benzyl alcohol; moderately soluble in water, ethanol, methanol, acetone, ethyl acetate and butyl acetate; and insoluble in ether, petroleum ether, carbon disulfide, benzene and amyl alcohol. It is stable at pH 2.0 after ten minutes at 100° but very unstable in alkali. Its bacterial spectrum is similar to that of aureomycin. Mice, injected intravenously, survived 6.25 mg./kg., and 60% of a second group survived 12.5 mg./kg. The luteomycin-producing strain, *Streptomyces* No. 144, is said to closely resemble *Streptomyces aureus,* of which it is considered to be a variant.

"MAGNAMYCIN"* (carbomycin). (See also "Ilotycin"). Molecular wt. approx. $C_{40}H_{67}NO_{16}$ from the data on a neutral equiva-

* Trademark name.

lent of about 860 and C, H and N analyses. Tanner et al. (207*a*) report that Magnamycin is a crystalline monobasic antibiotic recovered as slender, blunt-ended, needle-shaped crystals of the free base from which acid salts are readily prepared. The base melts with decomposition at 199.5–200.5° and is optically active; $[\alpha]_D^{25} = -58.6°$ in chloroform (c, 1%). A single absorption maximum in the ultraviolet region occurs at 240 mμ (9.4 mg. in 100 ml. ethanol). Characteristic absorption maxima in the infra red occur at 21 different wave lengths.

Although the base is only partially water soluble, the salts, i.e., sulfate or hydrochloride, are soluble in water. Magnamycin is very active against Gram-positive bacteria and shows activity against rickettsiae and psittacosis virus. Like Ilotycin, it is effective against Gram-positive organisms which are resistant to other antibiotics, and resistance toward Magnamycin by susceptible strains develops very slowly in step-wise fashion.

It is well tolerated by mice, cats, rabbits and dogs, regardless of the mode of administration. The LD_{50} intravenous dose of Magnamycin hydrochloride in mice is 550 mg./kg. Oral administration of Magnamycin was successful in treating patients suffering from infections caused by certain Gram-positive pathogens.

Although the antibiotic was reported as a product of several strains of *Streptomyces,* the one selected for this study (207*a*) was identified as *S. halstedii* (207*a*).

MANNOSIDOSTREPTOMYCIN. (See STREPTOMYCIN GROUP).

MICROCIN A AND B. Two antibiotics were recently isolated and studied by Taira and Fujii (205*c*). Extraction of crude culture liquors from ethyl acetate, first at pH 7.0 and then at pH 2.0, yielded two active fractions, named microcin A and B, respectively. Partially purified microcin A is a neutral reddish-purple powder, soluble in ethyl acetate and insoluble in water. Microcin B is an acidic yellowish-red powder, soluble in ethyl acetate and slightly soluble in water. Both substances gave a negative Molisch test and showed no coloration with ferric chloride. Microcin is active against Gram-positive bacteria and certain higher fungi but weakly so against some Gram-negative organisms and *Mycobacterium avium.* The LD_{50} dose in ten-gram mice is 6.25 mg./kg. The species of *Micromonospora* which produces the complex was not identified.

MICROMONOSPORIN. Studies on chemical analyses of preparations from culture filtrates (Waksman et al., 230, 232) indicated that micromonosporin is a highly pigmented and very unstable protein associated with a carbohydrate, the former having the solubility of an albumin. Extraction of the mycelium with organic solvents yielded a pigment with some of the characteristics of a quinone. It was suggested that the organism secreted a pigment in combination with a protein, whereas the mycelium contained the free pigment. The bacteriostatic spectra of the two substances were similar; each strongly inhibited *Bacillus subtilis,* was less active against Gram-positive cocci and *Bacillus mycoides,* and inactive against Gram-negative bacteria. The active substance was unstable below pH 3.0 or above pH 9.0. Heating at 100° for one hour caused a loss of two-thirds of the activity. The species of *Micromonospora* which produced the antibiotic was not determined.

MONAMYCIN. According to Thaysen (209), this antibiotic was named " monamycin " by Hassall, after Mona, St. Andrew, Jamaica. Information regarding it is limited to a preliminary report (59) wherein it is said to closely resemble the actinomycin group, although differing from any antibiotic of that group yet described. It is relatively stable and is effective against Gram-positive bacteria but inactive against Gram-negative forms. The green variant isolated from the original strain of Meredith's actinomycete (5) is said to produce monamycin by the surface culture method.

MUSARIN ($C_{35}H_{60}O_{14}N_2$). Musarin is a colorless high molecular weight acid with an equivalent weight of ca. 5000, discovered by Thaysen and Butlin (210) and subsequently studied by Arnstein et al. (5). The sodium or potassium salt is soluble in water, methanol, butanol and ethanol, but not in ether or acetone. The free acid is insoluble in 30% aqueous acetone and alcohol. The antibiotic is precipitated by mineral acids, barium or mercuric chlorides, and copper acetate. Musarin (sodium salt) decomposes at 170° without melting; is optically active, $[\alpha]_D^{20} = 35.1 \pm 1.6°$ (c. 1.21 in methanol), $[\alpha]_D^{20} = 38.7 \pm 2.7°$ (c. 0.736 in methanol), and absorbs ultraviolet light: $\lambda_{max} = 2400$, 2670 (inflexion) Å, $E_{1\,cm.}^{1\%} = 375$, 200 (in ethanol). Although the sodium salt is stable, the free acid is somewhat unstable, even as a dry solid. Exposures of 30 minutes at pH 2.0 or 11.0 at room temperature does not alter the activity, but 100° destroys it, either at high or low pH. At

pH 7.0 half of the activity remained after 30 minutes at 100°. Musarin is most effective against higher fungi but, in addition, shows considerable activity against Gram-positive bacteria and *Mycobacterium phlei*. Toxicity data have not been published. The antibiotic is produced in best yield by a red variant from Meredith's actinomycete. The name "musarin" refers to its ability to inhibit the growth of a fungus parasitic on the banana (*Musa sapientum*), not to the unidentified form of *Streptomyces* from which it is obtained.

MYCELIN. Unaware that Adámek et al. (1) had previously assigned the name "mycelin" to an antibiotic from *Hyphomyces*, Aiso et al. (1*b*) have reported that a second antibiotic named "mycelin" is formed in the mycelium of the culture which produces flavomycin (1*a*). Mycelin crystallizes in the form of prisms from acetone solutions. It turns black at 260° and decomposes at 263° (uncorrected). Sulfur or nitrogen have not been detected, and the antibiotic is Molisch-negative. It is soluble in chloroform, butanol, methanol, ethanol, amyl alcohol, acetone and benzene, and is insoluble in water, ether and petroleum ether. Mycelin in acetone solution is stable at pH 2.0, 7.0 and 8.8 for 24 hours and is also stable to heat in solutions at various pH values. It is active only against higher fungi, and the authors suggested that it might find use as a topical antifungal agent in medicine. The antibiotic is produced by *Streptomyces roseoflavus* (1*a*).

MYCETIN. This antibiotic is said to differ in certain respects from similar substances produced by *S. antibioticus* and Welsch's actinomycetin strain (Fainshmidt and Koreniako, 65). It is reported to be thermostable, soluble in water and extractable with benzene or chloroform but not with ether or petroleum ether. Since both active and inactive preparations are violet colored, it is not stated that the active substance, as such, is pigmented. One drop of an alcoholic concentrate in a dilution of 1 : 10,000 was said to inhibit the growth of *Staphylococcus aureus*. Later studies by Krassilnikov and Koreniako (124*a*) show that other Gram-positive bacteria and acid-fast types are inhibited, but Gram-negative organisms are unaffected. Certain strains tend to inactivate mycetin, among them *Pseudomonas fluorescens* and *B. radiobacter*. The mycetin-producing strain was reported to be *Actinomyces violaceus*.

MYCOBACIDIN (see ACTITHIAZIC ACID).

MYCOMYCIN ($C_{13}H_{10}O_2$), 3,5,7,8-tridecatetraene-10,12 diynoic acid (43). This unusual antibiotic, originally reported by Johnson and Burdon (114), has been chemically investigated by Celmer and Solomons (40, 42). It is a crystalline acid, m.p. 75° (decomp. explosively), $[\alpha]_D^{25} = -130°$ (c. 0.4, ethanol), and contains both acetylenic and allenic bonds in the molecule. Complete hydrogenation requires eight moles of hydrogen and gives *n*-tridecanoic acid. It is relatively stable at high dilutions (ca. 0.005 mg./ml.), but the half-life is inversely related to the concentration, and complete retention of activity is possible only by storage at −40° or lower. When mycomycin is treated with normal aqueous NaOH, the salt of an isomeric acid crystallizes from solution. Acidification of an aqueous solution gives the free acid "isomycomycin", $C_{13}H_{10}O_2$ (see Table IV) (41), which is optically inactive but, like the parent compound, gives *n*-tridecanoic acid upon catalytic hydrogenation. The original description (114) indicated that mycomycin was soluble in ether, amyl acetate and water. It is reported to be active against a wide variety of microorganisms, including acid-fast strains made resistant to streptomycin. Isomycomycin is about one-fourth as active as mycomycin in vitro against *M. tuberculosis* H37 RVR and shows very little activity against the mycomycin assay organism, *B. subtilis*. Crystalline material shows 50,000 units/mg. against *B. mesentericus* (*B. pumilus*) (42). The high activity of partially purified mycomycin against *M. tuberculosis* in mice (Hobby et al., 101) resulted in further efforts to stabilize the drug. A later report by Jenkins (112) indicated low toxicity of the factor when injected intraperitoneally into guinea pigs (more than 10,000 units per day for 35 days, with no ill effects), but the stability problem still remained. Anchel (3) has recently published on the acetylenic nature of antibiotics from Basidiomycetes, and Sörenson has compared the ultraviolet maxima of these compounds with polyacetylenic compounds from flowering plants of the family Compositae. Although none of these compounds appears to be identical with mycomycin or isomycomycin, they are markedly similar in composition and chemical properties. The mycomycin-forming strain has been identified as *Nocardia acidophilus* (40). This strain appears to be a new species of *Nocardia*. Its possible relationship, if any, to *Streptomyces acidophilus* was not reported.

NEAMINE. (See NEOMYCINS).

NEOMYCIN(S). Waksman and Lechevalier (233) originally reported neomycin to be a basic compound with a broad antibiotic spectrum and of special interest because of its activity against streptomycin-resistant bacteria. Later studies were made by Swart et al. (203, 204) on neomycin hydrochloride in pentasol-borate buffer on a Craig counter-current distribution machine. They presented evidence that neomycin is a mixture of three antibiotics and suggested that the term "neomycin complex" be substituted for neomycin. Peck et al. (167) isolated a fraction from neomycin which was a basic, water-soluble, nitrogenous substance, and designated it neomycin A. The amorphous hydrochloride, $[\alpha]_D^{25}$ +83° (c, 1.0 in water), showed 1700 units/mg. against *B. subtilis* by plate assay but only 50 units/mg. against *Escherichia coli*, turbidimetrically. Upon acid hydrolysis of neomycin, a substance showing about one-tenth the activity of the parent compound was obtained by Leach and Teeters (136). They assigned the name "neamine" to the compound and suggested $C_6H_{12-14}N_2O_3$ as a possible empirical formula. Further work by the same investigators (136*a*) proved that neamine and the neomycin A of Peck et al. (167) were identical and that the molecular formula might be a multiple of the C_6 compound. Independent investigations on the same problem were conducted by Dutcher and Donin (56). Regna and Murphy (177) and Dutcher et al. (58) isolated fractions, neomycin B and neomycin I, respectively, which apparently are identical. The latter workers also obtained another fraction, neomycin C ($C_{29}H_{58}O_{16}N_8$), which is isomeric with B. By comparing physical properties, "papergram" behavior and biological activities, it was found that their methanolysis product 1 (58), to which was assigned the empirical formula $C_9H_{19}O_5N_3 \cdot 3\,HCl$, is identical with Leach and Teeter's neamine and also with the neomycin A of Peck et al. (167). Although the identity of these products is apparent from their properties, the composition is not yet certain. Hydrolysis of neomycin A at 140° with hydrochloric acid yields a product with the composition of $C_6H_{14}O_5N_2$. It would appear, therefore, that the original product contains more than six carbon atoms and has either the C_9 composition or possibly the suggested C_{12} dimeric composition. As pointed out by Peck and Lyons (168*a*), the structures of the neomycins are similar to those of the streptomycins in that they contain an organic base in which amino-groups appear in place

of the guanido-groups of streptidine. In addition, there is a glycocidic fragment which varies in structure in neomycins A, B and C. Hamre et al. (87) recently found that neomycin A does not form colored complexes with either carbazole or anthrone reagent when treated with concentrated H_2SO_4 as do B and C. When the ninhydrin method is employed (for obtaining total neomycin activity) along with the color tests, the neomycin A and neomycin B plus C can be determined by difference.

Leach et al. (135) distributed neomycin sulfate in a 41-tube train using *n*-butanol, water and 2-ethyl butyric acid at pH 6.9. They obtained only one peak of activity instead of three, as reported by Swart et al. (204). In the latter work, Swart and coworkers recommended that the designation " neomycin B " be discarded, since it was in no way different from those substances commonly referred to as " neomycin " except that it lacked the neomycin A component. Leach et al. (135) found neomycin extremely stable toward alkali, since it withstood 18 hours of heating under reflux with excess barium hydroxide and was stable at pH 2.0 at room temperature for at least 24 hours. It is soluble in water, insoluble in common organic solvents. Acute toxicity tests in mice show an LD_{50} of approximately 80 mg./kg., intravenously, and 400 mg./kg., subcutaneously. The original neomycin-producing strain is closely related to *Streptomyces fradiae*, but other species of *Streptomyces* can also produce the complex.

NEONOCARDIN. Studies on a substance extracted from crude broths of a strain of *Nocardia* A.422 were made by Ueda and Uesaka (214*a*) and by Uesaka (214*b*, *c* and *e*). The crude hydrochloride, grayish-white in color, is prepared by a method of purification similar to that employed for streptomycin. Heating crude broths for 10 to 30 minutes at 100° produced no loss in potency; 90% was destroyed after 60 minutes at 100°. An increase in activity after 20 minutes suggested that more active material was released from the mycelium. It is soluble in water, methanol and ethanol; slightly soluble in amyl alcohol and benzene; and insoluble in ether, petroleum ether, toluene, amyl acetate and xylene. The antibiotic diffuses slowly through agar and is very active against Gram-positive cocci and certain Gram-negative pathogens but is somewhat less active against Gram-positive rods. Intraperitoneal

injection of two mg. of crude hydrochloride/mouse did not induce toxic reactions. Higher dosages were not tested because of a scarcity of the drug. The antibiotic is produced by a newly assigned species, *Nocardia kuroishi* (Uesaka, 214*d*).

NETROPSIN ($C_{32}H_{48}N_{16}O_4$). Analysis of crystalline netropsin sulfate indicates that it is a tetraacidic base, according to Finlay et al. (66). Netropsin sulfate melts at 224–225° and has a solubility of about 30 mg./ml. in water at 80°, but less than 0.5 mg./ml. at 25°. It is quite insoluble in the common organic solvents. The strong basicity of the antibiotic appears to be due to a substituted guanidine group, but this does not account for all of the nitrogen present. Crystalline netropsin hydrochloride was found to inhibit the growth of various Gram-positive and Gram-negative organisms and to be effective against clothes moth larvae and the black carpet beetle. The LD_{50} dose for mice is 17 mg./kg. intravenously and 70 mg./kg. subcutaneously. The antibiotic is produced by *Streptomyces netropsis* (66, 67) which is related to *S. reticuli* and *S. rubrireticuli.*

NIGERICIN ($C_{39}H_{69}O_{11}$). Nigericin is a high molecular weight acid whose crystalline sodium salt is obtained with relative ease from acidified broth filtrates. Harned et al. (88) found that the solubility of the salt or acid in water is less than 100 μg./ml., but both are freely soluble in ethyl, methyl and *n*-butyl alcohols. It is readily extracted from culture liquors with butyl acetate, ethyl acetate and ethyl ether. At neutral pH the antibiotic is stable in culture filtrates for two hours at 100°. The antibiotic spectrum includes activity against Gram-positive organisms, *Mycobacterium* and higher fungi. Potassium ions specifically inhibit the action of nigericin either in liquid dilution or on agar plate assays. The intraperitoneal LD_{50} dose in White Swiss mice is about 2.5 mg./kg. *Streptomyces* "Nig-1", which produces nigericin, has not been assigned a species name. This strain has been examined by Benedict and Lindenfelser (10) who found that it is closely related to two strains of *S. violaceoniger* which also produce nigericin-like antibiotics.

NITROSPORIN ($C_{20}H_{26}N_2O_6$). A white, crystalline, basic compound that has been studied by Umezawa and Takeuchi (221). The antibiotic becomes slightly brown at 115–120° and melts at

130–140° with decomposition. It is soluble in alcohol, ethyl acetate and acid water; slightly soluble in ether, benzene and ethylene-dichloride; and insoluble in water. Absorption maxima occurred at 2500 Å and 3200 Å. Upon exposure to air, the white crystals gradually change to a light brownish color. The antibiotic spectrum is relatively selective in that the growth of most Gram-positive rods and cocci are strongly inhibited and a majority of Gram-negative organisms are resistant. However, *Bacillus anthracis* and *Corynebacterium diphtheriae* are resistant, and the antibiotic inhibits group A hemolytic streptococci but not those of group C. The LD_{50} dose of nitrosporin in mice was 16 mg./kg., intravenously. The designation, *Streptomyces nitrosporeus,* has been assigned by Umezawa and Takeuchi to the nitrosporin-forming strain O-20.

NOCARDAMIN ($C_9H_{14}O_3N_2$). This unusual compound, containing a trimethyleneimine ring, was first reported by Stoll et al. (199) who obtained it in crystalline form and determined the structural formula (Stoll et al., 200) (see Table IV). The compound melts at 183–184° and is optically inactive. It is slightly soluble in water and the lower alcohols. Nocardamin is specifically active against various mycobacteria but shows no activity at a 1:1000 dilution against Gram-positive and Gram-negative bacteria. Toxicity data are not given. The strain of *Nocardia* which produces nocardamin is closely related to *N. flavescens* (199).

NOCARDIANIN ($C_{65\text{-}67}H_{96\text{-}104}O_{15}N_{18}$). Bick et al. (15*b*) isolated this rather complex factor from surface culture fermentations. The antibiotic crystallized from methanol as red prisms with m.p. 228–235° (decomp.); $[\alpha]_D^{25}$ −223° (C, 0.3 in methanol). Nocardianin is a weak base (Cram, 47*a*) and is readily soluble in chloroform, glacial acetic acid, and pyridine; moderately soluble in acetone and methanol; sparingly in water and ether; and almost completely insoluble in petroleum ether, carbon disulfide, and carbon tetrachloride. It is somewhat more soluble in dilute acids than in water. The ultraviolet absorption spectrum of a methanolic solution (C, 1.95×10^{-6} moles/l.) showed a minimum at 330 mμ (log E, 3.66) and a maximum at 440 (log E, 4.52). Negative tests were given for the following groups: alcohol (xanthine test), phenol, carbonyl, pyrrole, indol, peptide (biuret), and ester (hydroxamic acid test).

An alkali fusion experiment indicated the possible presence of pyrrole nuclei. The antibiotic is somewhat unstable in solution. It is active against Gram-positive bacteria. The species of *Nocardia* which produces the antibiotic has not been further identified (15*b*).

NOCARDIN. A water-soluble tuberculostatic substance called "nocardin" was isolated by Emmart (63) after extraction of the mycelium of an actinomycete with ether and 95% ethanol. A substance of similar nature was also present in the filtrates of the culture. Work conducted on the crude concentrates by Emmart et al. (64) showed that 25 mg. given twice daily to mice was equivalent to 3.0 mg. of streptomycin in suppressing the tuberculous process in mice, but that the toxicity of nocardin was considerably greater than that of streptomycin. The nocardin-producing strain seemed most closely to resemble *Nocardia coeliaca.*

PHAGOLESSIN A 58. In a search for agents active against bacterial viruses, Asheshov et al. (5*a*) isolated this substance as a light yellow hygroscopic powder, which gave negative biuret, Millon and ninhydrin tests, and a positive test for nitrogen. No crystalline derivatives were obtained with picrate, reineckate or helianthate although its properties suggest that it is basic in nature. It is soluble in acid water (pH 3.8) and methanol; less soluble in ethanol, and insoluble in acetone, ether, benzene, chloroform and petroleum ether. An aqueous solution at pH 3.0 could be boiled for 15 minutes without loss of activity, and was stable for several days at room temperature. Stability decreases as the pH rises, and at pH 9.0, there was an immediate, irreversible inactivation. The substance is weakly active against bacteria, with the exception of *Klebsiella pneumoniae,* which was inhibited at 3 μg./ml. Of 59 different bacteriophages and one actinophage, 37 were found to be sensitive to the antibiotic. The substance is quite toxic for mice, since intraperitoneal injections of 31.25 mg./kg. caused death to all of the test animals within 72 hours. The *Streptomyces* sp. which produces phagolessin A 58 has not been further classified.

PICROMYCIN ($C_{25}H_{43}O_7N$). (See PROACTINOMYCIN COMPLEX). This antibiotic precipitates from methanol as colorless well-formed crystals which melt at 169–170° (Brockmann and Henkel, 26, 27). Picromycin, so-named because of its persistent bitter taste, has basic

properties, contains no methoxyl groups and is optically active, $[\alpha]_D^{24} = 8.2°$ (ethanol c = 3.5); $[\alpha]_D^{24} = -50.2°$ (chloroform c = 6.3). Brockman et al. (*27a*) found that decomposition of picromycin by alkali yielded nearly one mole of dimethylamine and an oily distillable decomposition product. Chromic acid oxidation gave five moles of acetic acid. It shows no characteristic absorption in ultraviolet light; is readily soluble in acetone, benzene, chloroform and ethyl acetate; moderately soluble in ether and cold methanol; and difficultly soluble in carbon bisulfide, petroleum ether, water and aqueous alkali solution. It is stable to heating at 125° in vacuo for three hours. The bacterial spectrum, as reported by Suhren (201), shows strong activity against Gram-positive cocci and weak activity against *Escherichia coli*. The MLD for mice by the intravenous route is between 100 and 200 mg./kg. The original picromycin strain has been named *Streptomyces felleus*. A second, unidentified form of *Actinomyces*, superficially different from *S. felleus*, is also reported by Brockmann and Henkel to produce picromycin. Close similarity or possible identity of picromycin to the proactinomycin complex has been suggested (*27*).

PLEOCIDIN COMPLEX. Charney et al. (*45a*) have recently reported on two new streptothricin-like factors, pleocidin and pleocidin A. Pleocidin hydrochloride is a white hygroscopic powder, soluble in water, ethanol and methanol, and is most stable in aqueous solution at a range from pH 4 to 6. The *Escherichia coli/B. subtilis* ratios are 64 for pleocidin and 4 for streptothricin. The presence of pleocidin A, a rapidly diffusing but far less active entity, tended to complicate the recovery procedures. A satisfactory turbidimetric assay for pleocidin was developed by Fisher and Charney (*70a*). Like streptothricin, pleocidin is active against Gram-positive, Gram-negative, acid-fast organisms and higher fungi. The antibiotic is highly toxic for mice, and intraperitoneal injection showed an LD_{50} of 3 mg./kg. Orally the LD_{50} dose is about 40 times greater, indicating poor absorption from the intestinal tract. The pleocidin complex is produced by *Streptomyces* No. 272, which closely resembles *S. lavendulae* (*45a*).

PROACTINOMYCIN COMPLEX. Before the nature of this complex was realized, Gardner and Chain (*74*) had assigned the name "proactinomycin" to an organic base antibiotic which they had isolated from a species of *Proactinomyces*. According to Marston

(146), countercurrent studies conducted by Heatley (94) demonstrated that proactinomycin is composed of at least three antibacterial components. According to Marston's studies, the salient properties of these substances are as follows: proactinomycin A ($C_{27}H_{47}O_8N$) can be crystallized from wet ether as short thick plates or short prisms. Recrystallization attempts were unsuccessful, but A, containing a small amount of impurities, melted at 168–169°. Proactinomycin B ($C_{28}H_{49}O_8N$) was a doubtfully crystalline solid from pure " Pa B " obtained by addition of light petroleum ether to the homogeneous free base. The " crystals " melted at 83–87°. Proactinomycin C ($C_{24}H_{41}O_6N$) could not be crystallized. All three substances contain an $—N(CH_3)_2$ group and, like aureomycin and terramycin, yield dimethylamine on alkaline hydrolysis. The original substance was extractable into ether, amyl acetate, benzene and carbon tetrachloride; it could be reextracted back into water at pH 4.0. It was stable to ten minutes boiling at pH 2.0 or 7.0.

Marston and Florey (147) found that all three antibiotics inhibited the growth of Gram-positive cocci, *B. anthracis, C. diphtheriae* var. *gravis* and *N. meningitidis*. " Pa A " and " B " were equally active, while " Pa C " was about half as active as the other two. The approximate intravenous LD_{50} for 20-gram mice was 3.0 mg. for Pa A; 2.4 mg. for Pa B; and 1.6 mg. for Pa C. The complex is produced by *Proactinomyces* (*Nocardia*) *gardneri* (74).

RESISTOMYCIN ($C_{23}H_{18}O_6$). When recovered from the mycelium, processed and crystallized from dioxane, resistomycin was isolated in the form of saffron-yellow needles by Brockmann and Schmidt-Kastner (*29*). The crystalline compound decomposed around 315° without melting. It could be sublimed between 200–205° (10^{-4} Torr) without loss of activity and after heating for one hour at 250° showed no loss of antibiotic activity. No methoxyl or acetyl groups appear to be present, and a Zerewitinoff reaction indicated the presence of four active hydrogen atoms. In the ultraviolet, alcoholic solutions are intensely yellow; acetone and benzene solutions show a bright red fluorescence. The antibiotic is difficultly soluble in water and moderately soluble in ether, benzene, ethanol, acetone and glacial acetic acid. It is extremely stable to both acid and alkali. *Bacillus subtilis* and *Staphylococcus aureus* were inhibited at dilutions of 1 : 20,000,000, and five virulent strains of *M. tuberculosis* were inhibited at dilutions of 1 : 500,000 to

1 : 1,000,000. Believing their strain to be a new species, the above mentioned investigators have assigned to it the name *Streptomyces resistomycificus.*

RETICULIN (see Hydroxystreptomycin).

RHODOCIDIN. Charney et al. (45*b*) have stated that rhodocidin is a violet pigment which is soluble in water, methanol, ethanol and ether and insoluble in hexane. Activity is reported against a number of bacteria, including *Bacillus subtilis, M. pyogenes* var. *aureus, Proteus vulgaris* and *M. tuberculosis* H 37RV.

Mice infected with 1000 MLD of *Streptococcus* (species undesignated) were protected by injections of rhodocidin. The therapeutic index varied greatly with the route of administration. Although toxicity data were not presented in abstract (45*b*), there is an indication that the antibiotic is inactivated in vivo, and it appears that parenteral therapy is contraindicated. The *Streptomyces* species involved has not been further classified.

RHODOMYCETIN. Shockman and Waksman (192) have reported that rhodomycetin is a dark red powder, showing no definite melting point up to 300°. It does not appear to be an anthocyanidin derivative because of its very low nitrogen content, but may contain an anthraquinone nucleus. Like actinorhodine and litmodicin, it is red in acid solution and blue in alkali. The red form is soluble in ethylene glycol monomethyl ether, ethyl ether, acetone, acetic acid and the lower alcohols (methyl through amyl); it is insoluble in water. The blue form is water-soluble but extremely unstable in aqueous solutions. An alcoholic solution of the red form lost little activity when heated at 70° for ten minutes at a pH range from 4.5 to 9.3. It is active mainly against Gram-positive organisms and mycobacteria. Gram-negative bacteria are inhibited only by high concentrations. Mice withstood 120,000 units subcutaneously and 15,000 units intraperitoneally without ill effect. The highest potency material reported was 70,000 units/mg., but it is not stated whether this lot was used for toxicity tests. The rhodomycetin-producing mutant is a red variant of *Streptomyces griseus.* The parent strain from which the variant was obtained originally produced streptomycin.

RHODOMYCIN. The formula for the crystalline perchlorate is $C_{22}H_{30}O_{11}NCl$ (Brockmann et al., 30). Highly purified prepara-

tions were dark red and exhibited amphoteric properties (Brockmann and Bauer, 22). A large number of salts were prepared which included a water-soluble hydrochloride and phosphate and a water-insoluble picrate. The sodium perchlorate derivative melts at 198° (decomposition). This salt, soluble in alcohol, acetone and pyridine, is difficultly soluble in water. From its red color in butanol, chloroform or ether solutions, rhodomycin readily changes to blue in basic solution. Absorption maxima in benzene occur at 575, 542, 535 and 504 mμ. Countercurrent distribution studies (30) indicated two maxima, one at tubes 3–6 (rhodomycin A) and the other at tubes 20–24 (rhodomycin B), using a 25-tube instrument. Mild acid hydrolysis of rhodomycin leads to the isolation of a red insoluble crystalline decomposition product, $C_{15}H_{14}O_6$, which appears to be a naphthazarine derivative. This product inhibits the growth of *S. aureus* at a dilution of 1 : 300,000. A colorless, soluble hydrolysis product, basic in nature, containing a methylimide N was also found. The parent antibiotic is said to inhibit the growth of the same organism at a dilution of 1 : 50,000,000. Activity against other organisms and toxicity to lower animals were not stated. The *Streptomyces* isolate which produces rhodomycin has been named *S. purpurascens* (30).

RIMOCIDIN *. A complex antifungal antibiotic whose sulfate crystallizes from aqueous methanol as large fragile plates containing 7% water of hydration (Davisson et al., 52). When dried at 56° in vacuo, its sulfate analyzed as C, 57.65; H, 7.82; N, 1.81; and S, 2.03. It shows optical activity, $[\alpha]_D^{25}$ +75.2° (c, 1% methanol). The melting point of the hydrated salt is 151° (decomposition). In 80% methanol, Rimocidin shows ultraviolet absorption at 279, 291, 304 and 318 mμ. It is active only against higher fungi, including a number of animal pathogens. It is hemolytic for human and rabbit erythrocytes at 30 μg./ml. The acute intravenous LD_{50} of Rimocidin for mice is approximately 30 mg./kg. The antibiotic is produced in culture simultaneously with terramycin by *Streptomyces rimosus.*

ROSEOMYCIN. According to Ishida (110), a crystalline helianthate (decomposition point 211–216°) and a crystalline reineckate (decomposition point 114°) were obtained from the pure hydrochloride of roseomycin. It is a basic compound, belonging to a

* Trademark name.

class which Kuroya et al. (129) have labeled the "streptothricin I" group. Gram-positive, Gram-negative and acid-fast organisms are inhibited by the antibiotic, although it is much less active in vitro against *M. tuberculosis* var. *hominis* than streptomycin or streptothricin. Toxicity is reported to be exceedingly low, i.e., mice weighing 15 to 20 grams are able to withstand 20 mg. of roseomycin hydrochloride, either intravenously or intramuscularly, without ill effect. Production is by Ishida's strain No. 36 which has been classified as *Streptomyces roseochromogenus.*

ROTAVENTIN. Hosoya et al. (105*a*) isolated a crystalline antibiotic from the mycelium of a reticulin-producing strain. When crystallized from a mixture of water, methanol and benzene, rotaventin appeared as white thin leaves. It contains no nitrogen or sulfur (105*b*). It changes from white to yellow at 130° and melts with decomposition at 170–175°. It is soluble in methanol, ethanol, butanol, amyl alcohol and acetone and is insoluble in ether, petroleum ether, chloroform, benzene, ethyl acetate, amyl acetate, trichloroethylene, water, 5% aqueous solution of sodium bicarbonate, and acidic water at pH 2. It is not precipitated in methanol or ethanol by addition of phosphotungstic acid, flavianic acid, methyl orange, picric acid, lead acetate, or calcium, mercuric or barium chlorides. Upon addition of concentrated sulfuric acid, a red color develops.

Rotaventin is specifically active against various fungi and shows no activity against bacteria. Mice weighing 11 to 13 g. survived intraperitoneal injections of two mg. of rotaventin in peanut oil, but succumbed within 48 hours after receiving five or ten mg. No toxic effect was noted when ten-mg. doses were given subcutaneously. The same investigators (105*b*) found that the unidentified antifungal agent from *Streptomyces griseocarneus* (12) is apparently identical with the rotaventin produced by their reclassified variant of *Streptomyces rubrireticuli* (105*d*).

STREPTIN. Concentrates of this antibiotic could be prepared from broths in a manner similar to that for the preparation of streptomycin or streptothricin. In his book, Waksman (229) has classified streptin as a basic compound, soluble in water and insoluble in organic solvents. Woodruff and Foster (251), who first reported streptin, found that the antibiotic is related to streptothricin but is

much more active against staphylococci and micrococci than either streptothricin or streptomycin. Waksman et al. (235) found that the streptin strain is not inhibited by 56 μg./ml. of streptothricin. Tests against streptomycin- and/or streptothricin-fast strains were not reported. The strain of *Streptomyces* which produces streptin resembles *Streptomyces lavendulae* in morphological characteristics but is closely allied to *S. reticulus-ruber* in biochemical aspects.

STREPTOCIN. Studies on this factor by Waksman et al. (234) show that it crystallizes from 1% glacial acetic acid-methanol or from acetone as rosettes which have no absorption maxima between 2200 and 6750 Å. The antibiotic is soluble in water, ethanol, methanol and ether, but not in chloroform. The crystalline form is stable in a pH range of 2.0–11.0 and may be heated at 100° for 60 minutes without losing its activity. It is active against Gram-positive bacteria, mycobacteria and *Trichomonas vaginalis*. A major portion of this factor is found in the mycelium of the *Streptomyces griseus* strains that produce it, including Waksman's streptocin-producing strain No. 3533, streptomycin-producing strain No. 3496 and grisein-forming strain No. 3478 (Kupferberg et al., 127). A factor distinct from actidione and streptocin and highly active against Gram-positive bacteria, especially *Sarcina lutea,* is also produced by No. 3533. Kupferberg et al. (127) believe that this factor is the same as that of Schatz and Waksman (189) and of Goret et al. (80).

STREPTOLIN COMPLEX. The first member of this complex, streptolin, was first described by Rivett and Peterson (184). The most potent preparation cited was a crystalline helianthate of streptolin which was reconverted to streptolin hydrochloride, assaying 34,000 *Escherichia coli* units/mg. The similarities of antibiotic 136 and streptolin were emphasized by Bohonos et al. (19) and Peterson et al. (171). Further studies on the streptolin complex, indicating differences in the two complexes, have been made by Peterson and co-workers (171*a*). Whereas the crude broths from both *Streptomyces* No. 11 (streptolin complex) and antibiotic 136 (*Streptomyces* 136-B) each contained five components, those of the latter had substantial amounts of streptothricin. The reviewer has chromatographed purified antibiotic 136 and found that it contains at least three components, and, as emphasized by Peterson (171*a*),

its identity should not be confused with Rivett's crystalline streptolin helianthate. This material was optically active, $[\alpha]_D^{28}$ $-22°$ (c, 1.8). In its physical and chemical properties, streptolin is more closely related to streptothricin than to streptomycin. Later Larson et al. (134) prepared streptolin sulfate (43,000 units/mg.) from the crystalline reineckate. When the homogeneity of these products was studied by ion-exchange paper chromatography, streptolin sulfate was found to consist of two components, A and B. Elemental analysis of streptolin A sulfate (32,000 units/mg.) and helianthate are in good agreement with the emipirical formula, $C_{24}H_{45}O_{11}N_7$, for the free base. A sulfate prepared from the mother liquors of the first helianthate crystallization assayed 53,000 units/mg. Chromatography showed this to consist primarily of streptolin B. Upon acid hydrolysis of the streptolin sulfate of Larson et al. (134), Smissman et al. (193) separated the hydrolyzate chromatographically and found five major ninhydrin-positive fractions. In addition to ammonia, one fraction resembling an amino sugar was found to contain most of the reducing power of the total streptolin. The greater portion of streptolin A consisted of two fractions. One formed a hydrochloride and *p*-hydroxyazobenzene-p^1-sulfonate which on analysis suggested $C_4H_{8\text{-}10}O_3N_2$ as the empirical formula for the free base. The other is the six-carbon carboxylic acid containing two primary amino groups, which Carter has also found in streptothricin. The name " isolysine " has been suggested for this compound by van Tamelen and Smissman (224*a*), whereas Carter et al. (39*a*) have proposed the name " β-lysine ".

Peterson et al. (171) isolated from streptolin culture liquor a fraction B which appeared identical with streptothricin. The bacterial spectrum of streptolin is similar to that of streptothricin. In toxicity tests, all mice survived 0.09 mg., but 0.27 mg./20-gram mouse injected intravenously was fatal. The streptolin-producing strain, *Streptomyces* No. 11, has not been further identified.

STREPTOMYCIN GROUP

STREPTOMYCIN ($C_{21}H_{39}O_{12}N_7$), N-methyl-L-glucosaminidostreptosido-streptidine (see Table IV). This antibiotic was originally described by Schatz, Bugie and Waksman (188) and subsequently purified and chemically studied by many groups of organic

chemists. The trihydrochloride of streptomycin has been obtained in crystalline form as the dihydrate (monoclinic prisms showing birefringence) by Heuser et al. (96). The dihydrate gradually decomposes without melting. As the free base or salt of an inorganic acid, streptomycin is very soluble in water, but the inorganic acid salts are insoluble in most organic solvents. In stability studies by Regna et al. (179) it was found that temperatures at or below 28° and a pH range of 3.0–7.0 were most favorable, whereas first order irreversible inactivation took place outside that pH range. Streptomycin is characterized by a wide bacterial spectrum, including the ability to inhibit growth of Gram-positive, Gram-negative and acid-fast bacteria. Resistance to the antibiotic is readily developed, and several organisms have been found which require the drug in order to initiate growth (Miller and Bohnhoff, 154; Kushnick et al., 131). This property of drug dependence is unique among the antibiotics thus far described and is of value in determining whether an unknown antagonistic form of *Streptomyces* is elaborating any member of the streptomycin group. Earlier studies by the U. S. Food and Drug Administration on the toxicity of streptomycin, as reported by Molitor (156) for small animals, show that intravenous LD_{50} doses may range from 145–300 mg./kg. to 600–1150 mg./kg., subcutaneously. The original streptomycin-producing strain was identified as *Streptomyces griseus*. Both Kurosawa (128) and Kobayashi et al. (121*a*) reported the isolation of streptomycin from strains identified as *S. olivaceus,* and the former obtained a streptomycin-like factor from a culture of *S. poolensis*.

For several years streptomycin has been manufactured on a large scale. It is useful in combatting Gram-negative infections and finds its greatest application in the treatment of tuberculosis.

STREPTOMYCIN II. This antibiotic (Johnstone and Waksman, 115) has properties identical with those of streptomycin. The strain of *Streptomyces* producing the antibiotic was named *S. bikiniensis*.

MANNOSIDOSTREPTOMYCIN (Streptomycin B), $C_{27}H_{49}O_{17}N_7$ (see Table IV). This factor was first reported by Fried and Titus (71, 72). From a crystalline reineckate they obtained the white amorphous hydrochloride which decomposed at 179–182° (corrected).

Heuser et al. (96) prepared a crystalline trihydrochloride dihydrate of mannosidostreptomycin (hexagonal plates which are isotropic). It is optically active, $[\alpha]_D^{26.6}$ $-54.1°$ (1% in water). Perlman and Langlykke (170) found a mannosidostreptomycinase in streptomycin-producing cultures of *S. griseus* which induced hydrolysis at the point of attachment of the mannose moiety. This enzyme could decompose mannosidostreptomycin to yield streptomycin. The bacterial spectrum of the former is similar to that of streptomycin, but four to eight times as much is required to inhibit most test organisms. Toxicity tests by Rake et al. (173) in mice indicate that mannosidostreptomycin and streptomycin have approximately equal LD_{50} values on a weight basis. Whereas mannosidostreptomycin was only one-third as active as streptomycin on a weight basis in experimental *Salmonella schottmülleri* infections in mice, it appeared to be more active than the latter when comparison was made on a unit basis. Mannosidostreptomycin is produced by streptomycin-forming strains of *Streptomyces griseus*.

DIHYDROSTREPTOMYCIN ($C_{21}H_{41}N_7O_{12}$). This form of streptomycin is not made biologically but may be prepared from streptomycin by reducing the latter in the presence of Adams' platinum catalyst which results in the addition of one molecular equivalent of hydrogen per each molecular equivalent of streptomycin at pressures from one to four atmospheres (Peck et al., 168; Bartz et al., 8). The resulting reduced form of streptomycin is said to be more stable toward alkaline reagents and to have biological activity qualitatively and quantitatively comparable to that of streptomycin itself. There seems to be some controversy regarding earlier claims that less damage occurs to the eighth cranial nerve in humans when this form is used in prolonged tuberculosis therapy.

STREPTOTHRICIN ($C_{20}H_{36}N_8O_9 \cdot 3\,HCl$). First reported by Waksman and Woodruff (240) as the initial member of what is often referred to as " the streptothricin complex ". Detailed chemical studies by Carter et al. (38) indicate that some very interesting organic compounds are involved in the makeup of the molecule. The hydrolysis of streptothricin shows that it yields CO_2 (1 mole), ammonia (1.5–2.0 moles, depending on conditions) and three ninhydrin-positive substances, labeled A, B and C. Compound A is a diamino-C_6 acid ($C_6H_{14}N_2O_2$) which is isomeric with lysine.

β,ϵ-Diamino-*n*-caproic acid was one of two possible structures (39*a*), and synthesis of this acid through ornithine and careful comparison with compound A have shown that these two acids are identical. The trivial name β-lysine is suggested for this compound. This acid occurs also in streptolin, viomycin and probably in certain other unnamed antibiotics on which Carter and associates have worked. Compound B is an amino acid whose analytical data on the flavianate indicate an empirical formula of $C_6H_9N_3O_5$. Compound C analyzes fairly close to a diaminohexose but is not a simple amino sugar. Earlier work on preparations of streptothricin hydrochloride, containing 830 *B. subtilis* units/mg., by Peck et al. (169) shows that this salt is optically active, $[\alpha]_D^{25}$ – 51.3° (c, 1.4 in water). Crude streptothricin in culture liquors is said to be stable to 15 minutes heating at 100°. The antibiotic has a wide spectrum and inhibits the growth of higher fungi as well as Gram-positive, Gram-negative and acid-fast organisms. Early studies on the toxicity of streptothricin were made with materials varying markedly in purity. As pointed out by Molitor (156), the delayed onset of the toxic reaction to streptothricin is the outstanding toxicological difference between this antibiotic and streptomycin. The intravenous injection of 1200 units of streptomycin/20-gram mouse caused 20% mortality over a period of 12 days, whereas the same amount of streptothricin showed 20% mortality over five days, 70% over nine days and 92% by the 12th day. Robinson et al. (185) found that aqueous solutions of streptothricin containing 200 units/ml. or more caused marked inflammation when applied to the rabbit eye over a 30-minute interval. The antibiotic is produced by selected strains of *Streptomyces lavendulae* and by an unidentified species of *Streptomyces* (213). The cultures producing antibiotic 136 and streptolin also form streptothricin.

STREPTOTHRICIN BI AND BII. Umezawa et al (223) studied the antibiotics produced by two strains of *S. fradiae,* Nos. 117 and 260. The former strain produced a factor designated as streptothricin BII, and the latter, streptothricin BI. According to Maeda (145*a*), primary studies on these factors were reported before the first neomycin paper appeared. A more detailed report on the chemistry of these compounds (145*a*) indicated that they are isomeric, and the results of elemental analyses of the crystalline reineckates,

p-(*p'*-hydroxyphenylazo)-benzene sulfonates and picrolonates indicated approximately $(C_{10}H_{20}N_2O_8)_n$ as the composition of their bases. Although these formulae are not in agreement with the suggested $C_{29}H_{58}O_{16}N_8$ formula for neomycins B and C by Dutcher et al. (58), Maeda feels that the characteristics of streptothricin BI are almost identical with those of neomycin C, and those of BII with those of neomycin B. He points out that the optical rotation of streptothricin BI hydrochloride, $[\alpha]_D^{25} + 82°$ (C, 0.5% in water), is similar to that of neomycin C, $[\alpha]_D^{25} + 80°$; and that the rotation of BII hydrochloride is $[\alpha]_D^{25} + 49°$, as compared with neomycin B, $[\alpha]_D^{25} + 54°$. Earlier comparisons of the melting point of Umezawa's crystalline reineckate of BI did not agree with those obtained for the reineckates of neomycin B or C by Dutcher (57). The similarities of BI and BII to neomycins C and B, respectively, in the opinion of the reviewer, appear to outweigh the disagreements on analytical data.

STREPTOTHRICIN VI. Studies conducted on this factor by Hutchinson et al. (106*a*) suggested a compound closely related to but not identical with streptothricin. However, later countercurrent distribution studies by Swart (202) indicate its apparent identity with streptothricin.

SULFACTIN. Although this antibiotic has been crystallized and the formula $(C_{36}H_{55}N_{11}O_7S_4)$ is in good agreement with the analytical data, Junowicz-Kocholaty et al. (117) state that a lower molecular weight formula $(C_{27}H_{40}O_5N_8S_3)$ seems also possible. The melting point was found to be 245–275° (corrected, decomposition). Crystallized sulfactin is very soluble in chloroform, soluble in ethanol, ethyl acetate, dioxane and butanol, and less soluble in methanol. It is almost insoluble in water, ether, petroleum ether and benzene. The pure substance is stable in boiling alcohol, but crude culture boiled at pH 7 for 30 minutes loses half of its activity. Morton (157) found that 0.0254 μg. of crystalline material inhibited the growth of *Staphylococcus aureus* P210. The antibiotic inhibits the growth of many Gram-positive organisms and Gram-negative cocci but does not affect Gram-negative rods at 32 μg./ml. The LD_{50} intraperitoneal dose for white mice (17- to 20-gram) was 2.75 mg. The sulfactin-forming strain of *Actinomyces*, designated as R-30, was found to resemble *Actinomyces roseus* (*S. roseochromogenes*) in some respects.

TERRAMYCIN. The empirical formula originally proposed for this antibiotic by Regna and Solomons (178) was $C_{22}H_{24\text{-}26}N_2O_9 \cdot 2\,H_2O$. Later work by Pepinsky and Watanabe (169*a*) suggested $C_{22}H_{24}N_2O_9 \cdot HCl$ for the crystalline hydrochloride. After considerable preliminary work on the degradation products of terramycin (101*a, b, c;* 126*a;* 165; 166), the structural formula for terramycin has been worked out as shown in Table IV. Chemically speaking, it is 4,8-diketo-1-dimethylamino-2,5,6,10,15,17-hexahydroxy-15-methyl-1,4,5,8,15,16,17,18-octahydro-3-naphthacenecarboxamide. A shorter name, " oxytetracycline ", has been proposed by Stephens et al. (195*b*). The antibiotic is amphoteric and forms the crystalline hydrochloride and sodium salt (Finlay et al., 68). Crystalline terramycin has the following properties: melting point 185° (decomposition); optical rotation $[\alpha]_D^{25}$ – 196° (1% in 0.1 *N* HCl); soluble in methanol, ethanol, acetone and propylene glycol, and in water to the extent of 0.25 mg./ml. at 25° but insoluble in ether and petroleum ether. It is stable at room temperature over long periods at pH 2.0–5.0, the half-life at 37°, pH 7.0, being 26 hours. Ultraviolet absorption maxima (in 0.1 *M* phosphate buffer, pH 4.5) occur at approximately 247, 275 and 353 mμ, and characteristic absorption in the infrared region is also shown.

The antibiotic exhibits a broad spectrum, including the ability to inhibit the growth of Gram-positive, Gram-negative and acid-fast organisms, as well as rickettsiae. Inoculated in high concentrations into either the yolk sac or the allantoic cavity, terramycin inhibited influenza A virus (PR8) infection of the chick embryo when given one to three hours before or up to one hour after the inoculation of ten to 100 ID_{50} of the virus, according to Vinson and Walsh (227). Loughlin et al. (143) found that terramycin is therapeutically effective in the treatment of enterobiasis (pinworm infections) in man. The original terramycin-producing strain has been assigned the name *Streptomyces rimosus.* Kurosawa (128) has reported the isolation of a terramycin-like factor from a strain of *S. roseochromogenus.* Waksman et al. (241) and Kochi et al. (121*b*) have found that their strain No. 3560, resembling either *S. alboflavus* or *S. griseoflavus* and nearly identical with *S. rimosus,* also produces terramycin and Rimocidin in shaken flask cultures.

Terramycin, like the other so-called broad spectrum antibiotics Chloromycetin and aureomycin, is now produced in large quantities

and finds wide use in medicine; it is effective also as a feed supplement and has particular application in rations for baby pigs.

THERMOMYCIN. Crude thermomycin, first reported by Schöne (191), is extracted from culture broths with ether and dries as a yellow powder. When heated for 15 minutes at 75°, about one-fourth of the activity against *Corynebacterium diphtheriae* was lost, and at 100° for the same period all activity was destroyed. The antibiotic did not dialyse through a cellophane membrane against distilled water. A number of Gram-positive and Gram-negative pathogens were tested for growth inhibition by thermomycin. Of the types tested, it strongly inhibited only the growth of *Corynebacterium diphtheriae* (*gravis, intermedius* or *mitis*) and showed a weak effect against *Listerella monocytogenes*. Injection of 512 *C. diphtheriae* units (two ml.) of broth filtrate was non-toxic to white mice. The antibiotic is produced by the S (smooth) type of *Streptomyces thermophilus*. This is the only reported example of an antagonistic thermophilic form of *Streptomyces*.

THIOAURIN ($C_{14}H_{12}O_4N_4S_4$). Bolhofer et al. (19*a*) recrystallized crude thioaurin from acetone and obtained bright orange-yellow crystals which melted with decomposition at 179–181°. The ultraviolet absorption spectrum showed two peaks; one at 232 mμ (E_{mol} = 12,400) and the other at 370 mμ (E_{mol} = 31,900). A sample of thioaurin heated in hydrochloric acid gave a crystalline yellow dihydrochloride, $C_{12}H_{14}N_4S_4O_2Cl_2$ (m.p. 210–215°), which retained antibiotic activity. Oxidation of thioaurin with 30% hydrogen peroxide yielded a crystalline microbiologically inactive product, $C_{14}H_{12}O_8N_4S_4$. The pure antibiotic is reported to be relatively insoluble in water and in many organic solvents. The growth of various Gram-positive and Gram-negative bacteria is inhibited by the antibiotic, but, unlike thiolutin, it shows little activity against higher fungi. The LD_{50} intravenous dose of thioaurin in mice is 16 mg./kg. The species of *Streptomyces* which produces the antibiotic has not been identified.

THIOLUTIN ($C_8H_8N_2O_2S_2$, as reported by Celmer et al., 43*a*). The first report on thiolutin was that of Tanner et al. (206). Like aureothricin, it crystallizes as brilliant yellow needles which have no definite melting point but darken at about 255°. Tanner et al. (207) noted a characteristic absorption curve in the ultraviolet

region with maxima occurring at 384 and 314 mμ and a shoulder at 245 mμ. Characteristic absorption is also exhibited in the infrared region. It is fairly soluble in the lower alcohols, methyl isobutyl ketone, chloroform, glacial acetic acid, pyridine, and dimethylformamide; slightly soluble in ethyl ether, petroleum ether, benzene, and hexane; and sparingly in water (120 μg./ml.). It is very stable in acid solution and withstands heating for one hour at 100°. Like aureothricin, it is soluble in concentrated hydrochloric acid and turns from yellow to green during a 24-hour period. It exhibits activity against a wide variety of Gram-positive, Gram-negative and acid-fast organisms, and the higher fungi. Seneca et al. (191*b*) demonstrated activity against *Endameba histolytica* and against three hemoflagellates. The same investigators conducted toxicity studies which showed that the LD_0 in mice on subcutaneous injection is 5–10 mg./kg. and the LD_{50} is 25 mg./kg. The oral LD_0 is 10 mg./kg. and the oral LD_{50} is 25 mg./kg. It is nonhemolytic and exhibits no renal damage when injected subcutaneously into rats at levels of 40 mg./kg. for five days. Tanner et al. (206) found the antibiotic to be produced by certain strains of *Streptomyces albus*.

TRICHOMYCIN. Studies by Hosoya et al. (105*e*) have shown that purified trichomycin is a yellow amorphous powder which could not be obtained in crystalline form. It decomposes at 155°, and the color changes from yellow to brick red. Nitrogen, sulfur or halogens could not be detected. Addition of concentrated sulfuric or hydrochloric acid caused the formation of a blue color which gradually changed to violet. Trichomycin gave negative ferric chloride, ninhydrin, biuret, Fehling, Benedict, Molisch, Tollens and quinone reactions. Highly purified material showed absorption maxima at 286, 346, 364, 384 and 406 mμ.

The antibiotic becomes soluble in water, aqueous acetone and aqueous ethanol or methanol at a slightly alkaline pH; is partly soluble in butanol, ethanol, methanol and acetone; and is insoluble in ether, petroleum ether, ethyl acetate, amyl alcohol and water. Heating at 100° for 30 minutes at pH 7.0–8.5 causes no loss in potency, whereas five minutes at pH 2.0 completely destroys the antibiotic. It is active against various yeasts and fungi, and a concentration of 0.628 μg./ml. was lethal for *Trichomonas vaginalis* and *T. foetus*. Mice, weighing 13–15 gm., were unaffected by sub-

cutaneous doses of 2.5 mg., but 0.16 mg. injected intraperitoneally was fatal within 24 hours. A second factor, exhibiting activity against Gram-positive and Gram-negative bacteria, was noted in cross-streak plates. The trichomycin strain, *Streptomyces* H-2609, has been assigned the name *S. hachijoensis* (105*e*).

VINACTIN COMPLEX (see Viomycin). Mayer et al. (149) have shown that an actinomycete which they isolated is capable of producing, in a glucose peptone medium, a purplish substance active against staphylococci and moderately active against *M. tuberculosis*. Addition of beef extract to the medium suppresses most of the above antibiotic and yields a group of three colorless antibiotics which are more active against mycobacteria than against staphylococci. Vinactin A, according to Townley et al. (212), crystallizes from methanol, is optically active and decomposes at 253°. It is a peptide which contains guanidine and creatinine groups; acid and alkaline hydrolyzate analyses reveal the following amino acids: lysine, serine, alanine, glycine, glutamic acid and aspartic acid. Vinactin B decomposes at 240° and is similar to A, although quantitative differences are evident.

Although the data on the chemical analyses of vinactin A and viomycin are not in agreement, other evidence has accumulated to show that these two antibiotics are similar and possibly identical. Working with *Escherichia coli*, strain B/r, on gradient plates, Szybalski and Bryson (205*b*) isolated 15 subcultures, each resistant to a different antibiotic. When the cross-resistance relationships of each resistant strain was established against the 15 different antibiotics used, including viomycin and vinactin, these two antibiotics appeared to be identical from a microbiological point of view. Similar results were obtained by the same authors (205*a*) with resistant strains of *M. pyogenes* var. *aureus* and *Mycobacterium ranae*.

Vinactin C gives a positive test for creatinine and is believed to contain serine, sarcosine, glycine and an amino acid with an R_f close to that of aspartic and glutamic acids. Vinactin A protected mice against infections with *Klebsiella pneumoniae, B. anthracis* and *M. tuberculosis*, and was active in embryonated eggs infected with *Rickettsia typhi*. The development of resistance by strains susceptible to vinactin A is said to be difficult, and toxicities are claimed to be very low. The strain producing this complex has been assigned the name *Actinomyces vinaceus* (149).

VIOMYCIN ($C_{18}H_{31-33}N_9O_8$). This antibiotic was investigated independently by at least two groups, Finlay et al. (69) of Chas. Pfizer and Company and Bartz et al. (9) of Parke, Davis and Company. Haskell et al. (89) found that it is a strong basic polypeptide which, upon acid hydrolysis, yields carbon dioxide, ammonia, urea, L-serine, α-, β-diamino-propionic acid, an unidentified guanidine compound and a C_6 diamino acid (which Carter and associates have found in streptothricin and streptolin). The crystalline sulfate and oxalate of viomycin have been studied by Finlay et al. (69). Hydrated viomycin sulfate decomposes at 280° and is optically active, $[\alpha]_D^{25} = -32°$ (c, 1% in water). It is very soluble in water but virtually insoluble in most organic solvents. It is very stable in acid (approx. 25% of the activity remained after 11 days incubation of the sulfate in *N* HCl solution at 37°). It is active mainly against acid-fast organisms and shows less activity against Gram-positive and Gram-negative bacteria. In mice the intravenous LD_0 of viomycin sulfate is 176 mg. of the free base/kg. The viomycin-producing strain has been assigned the designation *S. puniceus* by Finlay et al. (69) and *S. floridae* by Bartz et al. (9). Routein and Hofmann (187*a*) have presented evidence to indicate that the Pfizer strain (69) is identical with *S. californicus,* ATCC 3312.

XANTHOMYCINS A AND B. These two antibiotics were isolated by Thorne and Peterson (211) and named xanthomycin A and B because of their yellow color. Rao and Peterson (174) converted xanthomycin A to the crystalline hydrochloride, and elemental analyses gave the empirical formula, $C_{31}H_{36}N_4O_8 \cdot 3\,HCl$, agreeing with the molecular weight (Rast) of 600 for the free base. It is an orange colored solid, exhibiting characteristic quinoid properties and containing four methoxyl groups, one methylimide group and no (or at most, only one) active hydrogen. Prolonged reaction shows the release of two primary amino groups. It is soluble in organic solvents and is dextrorotatory in aqueous solution. The ultraviolet absorption spectrum exhibits an intense maximum at 265 mμ and a weak one at 345 mμ. Acid hydrolysis gives ethanolamine, methylamine and ammonia in a ratio of 2 : 1 : 1. Separations of A and B were accomplished by Craig countercurrent distribution. A crystalline derivative of xanthomycin B has not been obtained. The purest preparations are yellow and resemble A in solubility properties. Peterson (171*a*) states that pure A tends

to change over to xanthomycins B and C. Picrates of both A and B have similar spectra and are of equal toxicity to mice. When a mixture of A and B (90 : 10, respectively) was boiled at pH 6.0 for 25 minutes, 90% of the activity was lost, but at pH 1.0 the same preparation retained 86% of its activity. Xanthomycin strongly inhibits the growth of many Gram-positive and Gram-negative bacteria but does not affect *M. tuberculosis* in dilutions as low as 1 : 2000. As little as 3.2 μg., or 1808 units of xanthomycin A hydrochloride, was toxic to mice. Expressed on the basis of units of antibiotic, the purest preparation of B was equally toxic. The xanthomycin-forming strain, *Streptomyces* No. 94, has not been further identified.

Xanthomycin-like factors have been reported also by Mold and Bartz (155) and by Hosoya et al. (103), the latter stating that their production strain resembles *S. rutgersensis*.

"X"-FACTORS. X-206 ($C_{46\text{-}47}H_{80\text{-}82}O_{13}$), as reported by Berger et al. (15*a*), is an acid which crystallizes as colorless prisms, m.p. 126–128°; $[\alpha]_D^{29}$ +15.0° (methanol, c 2). It shows no characteristic absorption in the ultraviolet; it is soluble in alcohols, esters, acetone, ether and petroleum ether, and is practically insoluble in water and in aqueous alkali. It is unstable in mineral acid and strongly alkaline solutions. The sodium, potassium, silver and barium salts have been prepared.

X-464 ($C_{25}H_{40}O_7$) is a white crystalline acid, m.p. 172–174° (decomposition), $[\alpha]_D^{27}$ +65.9° (methanol, c 2), reported by Berger et al. (15*a*). Although X-206 and X-464 appear distinctively different from a chemical point of view, Szybalski (205*a*) has reported that the two appear to be identical on the basis of cross resistance studies.

X-537A ($C_{34}H_{52}O_8$), reported by the same investigators, was originally extracted from the mycelium. This acid, recrystallized from ethanol, melts at 100–109°, optical activity, $[\alpha]_D^{26}$ −7.2° (methanol, c 1). It shows ultraviolet absorption maxima at 317 mμ and 249 mμ in isopropyl alcohol. It is soluble in organic solvents and insoluble in water.

All three antibiotics are active in vitro against certain Gram-positive bacteria and mycobacteria, but are inactive against Gram-negative bacteria and fungi. The LD_{50} toxic doses in mice for the three antibiotics are: X-206, 11 mg./kg., subcutaneously; X-464,

2.5 mg./kg. and X-537A, 40 mg./kg., intraperitoneally. The unidentified strains of *Streptomyces* producing these factors were isolated from soils obtained in three different states.

APPENDIX TO THE ALPHABETICAL LIST

In addition to those in the foregoing alphabetical list, there are many unnamed incompletely described factors which should also be cited.

Reilly (180) has described a red-brown water-soluble factor which is yellow in acid solution and purple in alkali solution. It is very stable to acid and is highly active against Gram-positive bacteria. The antibiotic is produced by *Streptomyces* sp., S.K.C.C. 1377.

Baram and Meyer (6) found that an unidentified form of *Streptomyces* produced an antifungal complex containing five active components.

Others include the lytic agent from *Streptomyces* 105 of Darpoux and Faivre-Amiot (51), active against Gram-positive forms and various Gram-negative plant pathogens; *Streptomyces* 211, antagonistic to various fungi, including *Pythium graminicola* (Meredith and Semeniuk, 153); antibiotic 91-6 of Oki and Hata (163); and a very active antifungal factor from a species of *Streptomyces* which inhibits the growth of many phytopathogens (Leben et al., 139). Later work by these investigators (139*a*) leads them to suggest the tentative name "helixin" for the antifungal product derived from *Streptomyces* A158. However, comparisons of the productive strains and the chemical properties and stabilities of the products show marked similarities between helixin and endomycin and suggest the possibility of their identity. Further studies are required to ascertain the degree of similarity. Since the status of helixin as an entity distinct from endomycin has not been established, the reviewer feels that it should not be placed in the alphabetical list. Other factors include antibiotic complexes formed by strains H-277 (Hosoya et al., 105), H-792 (104) and H-1205 (105*c*) and basic streptothricin-like antibiotics Nos. 24, 39, 259 and 120 (Seki) which were investigated by Kuroya et al. (130). Kurosawa (128) has reported three antibiotics from species of *Streptomyces* as follows: antibiotic No. 1 from *S. phaeochromogenus* strain 406; a crystalline anti-*phlei* factor from *S. albus* strain

15; and terramycin-like factors from two strains of *S. roseochromogenus*. Although no attempt has been made to isolate the antibiotic factor(s), Ark and Oswald (4*a*) found that 11 out of 40 strains of *S. scabies* formed agar-diffusible factors active against *M. pyogenes* var. *aureus*, *Verticillium albo-atrum*, and *Fusarium oxysporum*. *Escherichia coli* was not affected. Those which showed strong antibiotic activity were mutants from strains that originally were very pathogenic.

CLASSIFICATION OF ACTINOMYCETOUS ANTIBIOTICS

An arbitrary classification of named antibiotics from the actinomycetes, including the well characterized but unnamed "X" factors, is set forth in Table V. The classification is arranged somewhat differently than that proposed earlier by Waksman (229*b*) and includes many additional factors upon which information has become available. Some may feel that grouping of factors on the basis of color vs. uncolored is not justified, since the chemical nature, i.e., acid, base or neutral compound, is known for a majority of the colored substances. The key is offered as a matter of convenience. Although antibiotic 3510 has not been purified to the extent that it is known to be a red substance, its close relationship to grisein appears to justify its inclusion under the heading chosen for the latter.

ANTIBIOTICS AND NUTRITION

The first indication that unknown growth factors plus an antibiotic (streptomycin) were stimulatory to chick growth was reported by Moore et al. (159) in 1946. Ott et al. (164) found that crystalline vitamin B_{12} could replace, in promoting chick growth, the crude APF substances which Bird (17) and other animal nutritionists had found in liver extracts, fish meal and cow manure. About the same time, Rickes et al. (183) discovered that culture broths of various bacteria and actinomycetes, including *Streptomyces griseus*, contain vitamin B_{12} activity. Crystalline vitamin B_{12}, isolated from *S. griseus* fermentations, was found to have the same growth-promoting and anti-anemia activity as that derived from liver. Thus spent *Streptomyces* fermentation beers became very important sources of crystalline B_{12} for clinical use and of B_{12} supplements for animal nutrition. When Stokstad et al. (197)

TABLE V

CLASSIFICATION OF ACTINOMYCETOUS ANTIBIOTICS

A. Pigmented Substances
  I. Yellow to greenish-yellow to orange
    1. Low solubility in water, insoluble in ether, soluble in other organic solvents
      a. Golden yellow base, $C_{22}H_{23}O_8N_2Cl$, broad spectrum, acid stable, low toxicity ..... Aureomycin
      b. Yellow amphoteric compound, $C_{22}H_{24}O_9N_2$, broad spectrum, acid stable, low toxicity ..... Terramycin
      c. Weak base, greenish-yellow, $C_{36}H_{54}N_4O_4$, strongly antifungal ..... Fradicin
    2. Practically insoluble in water, slightly soluble in ether, soluble in other organic solvents
      a. Yellowish powder, active against yeasts and higher fungi ..... Actinone
      b. $C_9H_{10}N_2O_2S_2$, golden yellow needles ..... Aureothricin
      c. Neutral compound, brilliant yellow, $C_8H_2N_2O_2S_2$, wide spectrum ..... Thiolutin
      d. $C_{14}H_{12}O_4N_4S_4$, orange-yellow crystals ..... Thioaurin
      e. Yellowish-orange bases, $C_{21}H_{28}N_4O_8 \cdot 3\,HCl$ (A), highly active and extremely toxic ... Xanthomycins A and B
      f. Weakly acidic, $C_{28}H_{16}O_8$, saffron-yellow crystals, markedly stable to acid, alkali, and heat, mainly anti-Gram-positive ..... Resistomycin
    3. Soluble in water and in organic solvents other than ether
      a. Yellowish-orange crystals, wide spectrum, acid stable ..... Luteomycin
      b. Neutral yellowish-green pigment, anti-Gram-positive ..... Actinomyceline
      c. Yellowish crystalline powder, weakly acidic; active mainly against yeasts and higher fungi ..... Flavacid
    4. Insoluble in water and ether, soluble in other organic solvents
      a. Acidic yellow needles, $(C_{8.6}H_{7.6}NO_3)_x$, acid stable, mainly anti-Gram-positive ..... Griseolutein
      b. Yellow amorphous powder, antifungal and trichomonadicidal ..... Trichomycin
  II. Yellowish red to dark red, blue and purple. All members of this group, with the exception of grisein, the grisein-like factor, and the microcins, are primarily anti-Gram-positive
    1. Practically insoluble in water, slightly soluble in ether and soluble in other organic solvents
      a. Red cyclic peptide, $C_{40}H_{57}O_{11}N_7$ containing D-allo-isoleucine, very toxic ..... Actinochrysin
      b. Red cyclic peptide, $C_{41}H_{59}O_{11}N_8$ allo-isoleucine absent, very toxic ..... Actinomycin
      Strongly resembling actinomycin, possibly identical ..... { Actinoflavin / Actinomycin J_1 }
    2. Acid-base indicators, changing from red to blue, possibly quinones or anthocyanins
      a. Non-nitrogen-containing quinone, $C_{22}H_{20}O_{10}$, alkali-soluble, ether-insoluble ..... Actinorhodine

b. Anthocyanin-like pigment, soluble in water and ether ..... Litmocidin
c. Low nitrogen-containing compound; red form, water-insoluble, ether-soluble ..... Rhodomycetin
d. Amphoteric substituted quinone(s), crystalline perchlorate, $C_{22}H_{30}O_{11}NCl$, low water solubility Rhodomycins A and B
e. Reddish-purple pigment, active against Gram-positive bacteria ..... Coelicolorin

3. Red color due to a metallic element
a. Weak acid, containing iron and amino acids, $C_{40}H_{61}N_{10}O_{20}SFe$, soluble in water and phenol ..... Grisein
b. Impure, grisein-like factor, production influenced markedly by iron, narrower spectrum than grisein Antibiotic 3510

4. Non-metallic factor; possibly containing pyrrole nuclei
a. Red crystalline compound; weak base, $C_{35-47}H_{96-104}O_{15}N_{18}$. Sparingly soluble in water and ether, anti-Gram-positive ..... Nocardianin

5. Chemically unclassified violet pigment
a. Soluble in ether, water, and alcohols; antibacterial ..... Rhodocidin

6. Partially purified compounds
a. Neutral, reddish-purple powder; antifungal and antibacterial ..... Microcin A
b. Acidic yellowish-red powder; antifungal and antibacterial ..... Microcin B

B. Non-pigmented Substances
1. High molecular weight acids, containing nitrogen
a. Weakly acidic phenol, $C_{28}H_{40}O_9N_2$, highly fungicidal and insecticidal, insoluble in water, soluble in ether and other solvents ..... Antimycin A
b. Probable complex of factors related to antimycin A, tentative designation ..... Antimycin B
c. Crystalline acid, $C_{25}H_{43}NO_6$, active against cocci and certain spirochaetes, soluble in ether and other solvents ..... Borrelidin
d. Acid-form soluble in alcohols and methyl cellosolve, insoluble in water, ether, and other solvents; heat stable, antifungal and antibacterial ..... Endomycin and Helixin
e. $C_{28}H_{39}O_{14}N_3$, acid-form soluble in alcohol, insoluble in water and ether, antifungal and antibacterial, unstable to heat ..... Musarin

2. High molecular weight acids containing no nitrogen
a. $C_{28}H_{46}O_{11}$, practically insoluble in water, soluble in ether and other solvents, antifungal and antibacterial .. Nigericin
b. $C_{46-47}H_{80-82}O_{12}$, practically insoluble in water, soluble in ether and other solvents, mainly anti-Gram-positive X-206
c. $C_{28}H_{40}O_7$, same characteristics as above X-464
d. $C_{34}H_{52}O_8$, same characteristics as above X-537A

3. Weakly acidic, containing unique glutarimide ring
a. $C_{17}H_{23}NO_4$, soluble in water, chloroform, and ether, highly antifungal ..... Acti-dione

4. Weakly acidic, containing unique trimethyleneimine ring
a. $C_9H_{14}N_2O_2$, primarily active against mycobacteria ... Nocardamin

5. Vitamin analogue of biotin
   a. $C_9H_{15}O_3NS$, primarily active against mycobacteria, activity reversed by biotin ..... Actithiazic Acid
   b. Identical with the above compound ..... Mycobacidin
6. Highly unsaturated and easily decomposed acids
   a. $C_{12}H_{10}O_2$, wide spectrum ..... Mycomycin
   b. $C_{12}H_{10}O_2$, isomeric with mycomycin ..... Isomycomycin
7. Antibacterial and antiviral compound
   a. White, acidic antibiotic, selective spectrum and high toxicity ..... Cardicin

C. Non-pigmented Organic Bases
1. Relatively complex compounds, some of which are known to contain amino sugars; soluble in water, insoluble in organic solvents
   a. $C_{21}H_{39}O_{12}N_7$, yielding maltol on alkaline hydrolysis, wide spectrum, low toxicity ..... Streptomycin
   b. $C_{21}H_{39}O_{13}N_7$, yielding isomaltol on alkaline hydrolysis, wide spectrum, low toxicity Hydroxystreptomycin and Reticulin
   c. $C_{27}H_{49}O_{17}N_7$, enzymatic cleavage yields streptomycin and D-mannose; shows lower biological activity than streptomycin ..... Mannosidostreptomycin
   d. $C_{21}H_{41}O_{12}N_7$, chemically reduced streptomycin Dihydrostreptomycin
2. Relatively complex compounds, soluble in water and insoluble in organic solvents, some of which are known to contain amino acids, amino sugars and other unidentified fragments
   a. $C_{20}H_{36}N_8O_9 \cdot 3\,HCl$, containing two amino acids and one amino sugar, wide spectrum, delayed toxicity .. Streptothricin
   b. Compounds similar to but not identical with streptothricin ..... Pleocidin and Pleocidin A
   c. $C_{24}H_{42}O_4N_7$, unidentified fragments plus an amino acid identical with one of those contained in streptothricin ..... Streptolin A
   d. Similar to streptolin A ..... Streptolin B
   e. Closely related to streptolins ..... Antibiotic 136
   f. Chemically unidentified, biologically similar to streptothricin ..... Actinorubin and EI_5
   g. Same characteristics as above ..... Lavendulin
   h. Same characteristics as above but shows greater activity against cocci ..... Streptin
3. Compounds with limited chemical data, one complex apparently related to streptomycin and streptothricin, wide spectrum
   a. Undegraded, basic complex ..... Neomycin
   b. $C_9H_{19}O_5N_3$ or $C_{12}H_{26}N_4O_5$? ..... Neomycin A Synonym, neamine
   c. $C_{23}H_{46}O_{13}N_6$ ..... Neomycin B
   d. Similar to neomycin B ..... Catenulin
   e. $(C_{10}H_{20}N_2O_5)_n$ closely related to neomycin B .. Streptothricin BII
   f. $C_{23}H_{46}O_{12}N_6$, but degradation products differ from those of neomycin B ..... Neomycin C
   g. $(C_{10}H_{20}N_2O_5)_n$ closely related to neomycin C ... Streptothricin BI
   h. Closely related to the neomycins ..... Flavomycin
   i. Sakaguchi negative, maltol negative compound, probably related to the neomycins ..... Dextromycin

j. Limited activity against *M. tuberculosis;* low toxicity ..... Roseomycin

4. Basic polypeptides which contain guanidino and/or creatinine groups
   a. $C_{18}H_{31-38}N_9O_8$, containing a guanidino compound but no creatinine group, fairly low toxicity ..... Viomycin
   b. Compound indistinguishable from viomycin in cross-resistance patterns ..... Vinactin A
   c. Closely related to vinactin A ..... Vinactin B
   d. Compound containing creatinine group but no guanidino group ..... Vinactin C
   e. Polypeptide containing a guanidino group active against Gram-positive rods, but not against Gram-positive cocci ..... Cinnamycin
5. Miscellaneous basic compounds whose chemical relationships to substances previously classified are unknown
   a. $C_{24-26}H_{60-65}NO_{12-14}$, crystalline base, biologically more similar to penicillin than to aureomycin, terramycin, and chloramphenicol; limited effect on Gram-negative rods and low toxicity ..... "Ilotycin" (Erythromycin)
   b. Approx. $C_{40}H_{67}NO_{16}$, crystalline monobasic substance, active against Gram-positive bacteria, and rickettsiae—very low toxicity ..... "Magnamycin" (carbomycin)
   c. $C_{28}H_{48}N_{12}O_4$, a water-soluble, solvent-insoluble, tetra-acidic base containing a substituted guanidino group, active against certain bacteria and insects ..... Netropsin
   d. $C_{20}H_{28}N_2O_6$, water-insoluble, solvent-soluble, selective activity against Gram-positive bacteria ..... Nitrosporin
   e. $C_{37}H_{47}O_5N$ (A), $C_{35}H_{45}O_5N$ (B), and $C_{34}H_{41}O_5N$ (C), related series, soluble in organic solvents, selective antibacterial action. One factor possibly identical with picromycin ..... Proactinomycins A, B, and C
   f. $C_{25}H_{43}O_7N$, practically insoluble in water, soluble in organic solvents, active mainly against Gram-positive bacteria ..... Picromycin
   g. Basic substance, soluble in water, active against various Gram-positive and Gram-negative bacteria .... Neonocardin
   h. Basic compound, selective spectrum, effective against *Trypanosoma equiperdum* ..... Achromycin
   i. Amorphous hydrochloride, active primarily against acid-fast organisms ..... Anti-phlei factor
   j. Basic compound, weakly antibacterial; active primarily against bacteriophages ..... Phagolessin A58

D. Non-pigmented neutral compounds
1. Soluble in water, ether, and other solvents
   a. $C_{11}H_{12}N_2O_5Cl_2$, broad spectrum and low toxicity .. Chloromycetin
2. Nitrogen-free, crystalline antifungal agent ..... Mycelin
3. Complex compound containing both basic and acidic groups
   a. Crystalline antifungal agent showing no activity against bacteria ..... Rimocidin

E. Miscellaneous group, specific chemical nature (i.e., acid, base or neutral compounds) unknown, or not reported
   a. Sulfur-containing compound, almost insoluble in water and ether but soluble in other organic solvents, highly active against Gram-positive bacteria .... Sulfactin
   b. Crystalline factor, soluble in water and ether, insoluble in chloroform, active against Gram-positive bacteria and *Trichomonas* ..... Streptocin

1. Specifically active against higher fungi
   a. Strongly reducing compound, soluble in the lower alcohols ..... Fungicidin
   b. Differing slightly from fungicidin in antifungal spectrum ..... Fungicidin RAW
   c. Crystalline factor; UV absorption spectrum identical with b., slightly different antifungal spectrum ..... Chromin
   d. Mixture of four antifungal factors ..... Cacaomycetin
   e. White needles, containing no nitrogen, strictly antifungal ..... Rotaventin
   f. Partially purified factor, similar to fungicidin in some respects ..... Ascosin

F. Incompletely described
1. Proteins or polypeptides?
   a. Lytic effect on both live and dead bacteria ..... Actinomycetin
   b. Antibiotic combined with orange pigment, active against Gram-positive bacteria ..... Micromonosporin
2. Active against mycobacteria
   a. Thermostable, active only against acid-fast strains Anti-smegmatis factor
   b. No activity against other forms reported ..... Nocardin
3. Active against Gram-positive bacteria
   a. Related to the actinomycin family ..... Monamycin
   b. Possibly related to actinomycetin ..... Mycetin
   c. Probably closely related to mycetin ..... Actinomyces lysozyme
   d. Anti-*Corynebacterium* factor ..... Thermomycin
4. Active against certain viruses
   a. Active only against certain viruses ..... Ehrlichin
   b. Antibacterial and antifungal effect very low; active against eastern and western strains of equine encephalomyelitis virus ..... Abikoviromycin

noted a growth response over and above that attributable to the B_{12} content of their aureomycin fermentation residues, they reasoned that it was due to the antibiotic. Subsequently they were able to prove that this, indeed, was true (198). Since that time a voluminous literature has developed on the subject of growth stimulation of non-ruminant animals. To mention but a few of the many contributions on this subject, one might cite the work of McGinnis et al. (150) in demonstrating the growth-stimulating effect of aureomycin plus B_{12} for turkey poults; the work by Whitehall et al. (249) with chicks; and that of Carpenter (36) and of Leucke et al. (141) with swine. Subsequent to the discovery that aureomycin possesses growth-stimulating properties, terramycin was found to be equally effective, and Magnamycin is reported to be active in stimulating chick growth (207*a*). Streptomycin is somewhat less active, and pure Chloromycetin appears to be the least effective of this group. In addition to these antibiotics of actinomycetous origin, bacitracin and penicillin, of bacterial and fungal origin, respectively, were found to be highly effective.

Supplementation of feed for an eight-week period with a vitamin B_{12}-antibiotic combination often produces weight gains in young chicks and turkey poults up to 30% greater than that of control birds. Carpenter (36) found that young pigs gained up to 0.88 pound per day on a diet supplemented with B_{12} and 1.25 grams of terramycin per 100 pounds of feed, whereas the controls gained only 0.57 pound per day. These same animals gained one pound for each 3.02 pounds of feed consumed, but the controls required 4.12 pounds of feed to show the same gain.

An interesting new development in the raising of baby pigs has been reported by Luther (144) who developed a synthetic sow's milk, comprised primarily of skim milk, lard, corn oil, fish solubles, emulsifiers, dried brewers' yeast, dried blood meal, vitamins, minerals and terramycin (10–50 gm./ton). A similar "milk" enriched with aureomycin is available. It was found that baby pigs could be separated from the sow after two days and, when raised under carefully controlled conditions, could be brought to weights of 40.9 pounds at eight weeks and 113 pounds at 105 days compared with weights of the sow-fed control pigs of 28.1 pounds and 86 pounds, respectively. Some of the advantages of this procedure include (*a*) a decrease in baby pig mortality, (*b*) three litters per year as against one or two larger litters, and (*c*) lactation is eliminated as a controlling factor. The nutrition of young colts, dogs, mink and sheep are reported materially improved by feeding such antibiotic-containing "milks".

Blight (18) has pointed out that antibiotics are not always interchangeable on an equal weight basis, i.e., it may require up to ten times as much of one antibiotic as it does of another to produce the same growth response. Comparative studies of several antibiotics, individually evaluated in the same experiment, may show that some are highly active and others are ineffective. Factors which Blight feels may influence the results are the general type of diet being used, the condition and age of the animals at the time of supplementation, and the presence of specific disease-producing organisms. Many antibiotics remain to be tested for growth-promoting abilities. Future work may show some of these to be even more effective than the medically useful group which has received the most attention to date.

In some unknown manner, antibiotics appear to exert a sparing effect upon some feed nutrients. Biely and March (16) reported

no significant improvement of growth in chicks fed a simplified ration composed mainly of ground yellow corn, casein, gelatin, and vitamin and mineral supplements, fortified with aureomycin. All of the known B-group vitamins were present. When riboflavin, niacin or folic acid was removed from the ration, retarded growth was observed. When aureomycin was included in the deficient ration, growth was resumed. Matterson and Singsen (148) found that the greatest percentage growth response in chicks is obtained on the poorer quality rations (all plant protein), and that the better the quality of the ration with respect to protein (especially of animal origin) and vitamins, the less is the growth response obtained by supplementing the ration with antibiotics.

Numerous explanations have been given to account for the manner in which antibiotics function as growth stimulators. Those advanced by Norris (161) include the following: (*a*) Antibiotics may change the character of the microflora of the intestinal tract so that types which synthesize essential nutrients are better maintained; (*b*) they may destroy microorganisms which compete for the available supply of essential nutrients in the diet; (*c*) they may prevent the development of a mild chronic enteritis which interferes with the absorption of essential nutrients; and (*d*) they may influence certain biochemical systems so that increased synthesis of essential nutrients is obtained without greatly altering the character of the microflora. To this list Blight (18) has added two more possibilities: (*a*) That antibiotics may reduce moisture content of the intestinal material, which then passes along more slowly, thus permitting more efficient utilization, and (*b*) that certain antibiotics may possibly have hormone-like properties. Weber et al. (243) feel that low concentrations of antibiotics may increase intestinal protein imbibition. This theory suggests that the proteins forming the intestinal wall would then take up increasing amounts of water from the intestinal tract along with the various crystalloids which normally pass from the digestive tract to the blood stream. Increased growth may result from an increased rate of nutrient absorption from the intestinal tract, favored also by a possible lower general level of disease.

Although not strictly within the scope of this review, surface-active agents are beginning to challenge the growth-stimulating effects of antibiotics. For example, Leucke et al. (142) found that when ethomid C/15, an *N*-substituted fatty acid amide, was fed at

a level of 0.1% to hogs, the average daily gain in weight was 1.29 pounds as compared with 1.28 pounds for the same diet containing five mg./lb. of aureomycin.

For a number of reasons it is difficult for the reviewer to favor the change of flora concepts: (*a*) Aureomycin and terramycin, and to a lesser degree streptomycin, are all broad-spectrum antibiotics, whereas penicillin and bacitracin would tend to influence only the growth of Gram-positive strains; and polymyxin, which has a stimulatory effect in chicks (169*b*), inhibits primarily the Gram-negative forms. It does not appear that, to obtain results, one must necessarily alter the growth of a specific group or groups, i.e., Gram-positive or Gram-negative, or both. (*b*) The use of the antibiotic, Chloromycetin, is interesting. It depresses the growth of the same type of organisms as aureomycin and terramycin. If growth stimulation is due only to specific changes in microflora, one might reason that it should be as efficient as the other two in growth-promoting capacities. This, however, is contrary to the findings of Wallace et al. (242); Chloromycetin failed to stimulate the growth of swine fed a corn-peanut meal ration, whereas significant growth responses were obtained with aureomycin and terramycin. Employing the same type of animals, Carpenter (37) noted a slight growth gain with Chloromycetin, but it was the least effective among five antibiotics which he tested. Branion and Hill (19*b*) found that Chloromycetin at levels of 25 mg./kg. of diet did not improve the growth or feed efficiency of turkey poults, either on an animal protein diet or a plant protein basal diet supplemented with vitamin B_{12}. However, Yacowitz et al. (251*b*) have found that crude Chloromycetin added to a chick ration increased growth up to 23% above those fed a control ration containing 18 μg./lb. of vitamin B_{12}, whereas the crystalline drug (1.48 mg./lb.) caused only a 5% growth increase. They found that, in contrast to crude Chloromycetin, aureomycin, terramycin and procaine penicillin G, crystalline Chloromycetin is rapidly adsorbed from the duodenum of the chick, and they postulated that too little is left to affect the microflora in the lower portion of the gastrointestinal tract.

(*c*) Certain antifungal agents, including at least one of *Streptomyces* origin (Rimocidin), have been shown to have growth-stimulating properties for chicks. Norris' fourth theory and that of Weber et al. (243) would seem to contain, therefore, the fewest number of loopholes, although it is readily admitted that one is

confronted with a complex situation for which there is no easy solution. The experiments of Branion and Hill (19*b*) suggested that the mode of action of the stimulatory antibiotics is even more complex than some of the theories advanced would indicate.

In response to many requests, Reyniers, Luckey and Gordon (180*a*) at the Lobund Institute, University of Notre Dame, have conducted preliminary experiments on the effect of feeding various sterile antibiotics and a sulpha drug to germ-free chicks and turkey poults. There was no indication of an added growth response in germ-free chicks whose diets were supplemented with streptomycin, bacitracin or Chloromycetin at levels ranging from 23 to 50 mg./kg. and sulphasuxidine at 2000 mg./kg. The results with terramycin and procaine penicillin were somewhat variable, but in general represented a negative response. Conventional birds from the same clutches which were fed the same sterilized diet containing sterile antibiotics gave positive growth responses.

In an over-all analysis of the results, these are some of the salient facts which were pointed out: (*a*) Germ-free birds grow much faster than conventional birds fed a diet containing no stimulatory antibiotic; (*b*) conventional birds receiving growth-promoting antibiotic have some of the attributes of germ-free birds and show thymic stimulation and "gastrointestinal depression"; and (*c*) selected maximum body weight, germ-free birds show indications of thymic stimulation without gastrointestinal depression. The growth effect of antibiotics in the conventional animal may have a rather complex point of attack. Reyniers emphasized the fact that when antibiotics are administered as growth promotants to conventional birds, there is little or no change in the intestinal flora; yet almost all of the characteristics of the germ-free state are present. He does not believe that the mode of action is explainable in terms of the microbial flora, but instead that the phenomenon lies outside the range of continued microbial interference and within the physiological mechanisms of the host which have been activated by the initial contamination. The experiments thus far conducted have served to emphasize the complexity of the problem and suggest various lines of endeavor which may be pursued in attaining its successful solution.

For each advantage gained, there often crops up a disadvantage. Fortunately the case to be cited is one which affects relatively few

individuals. Greiff and Pinkerton (81) have found that the introduction of antibiotic mash containing aureomycin or terramycin into the diet of a flock of laying hens resulted in progressively increasing resistance of the eggs to rickettsial growth, the resistance becoming almost complete at the end of three weeks, hence rendering them unusable for assaying anti-rickettsial activity. No effect on the multiplication of influenza A virus was observed.

The medically-useful antibiotics have been helpful to the farmer in the treatment of many animal diseases, particularly mastitis in dairy cattle. However, as Jezeski (113) points out, these wonder drugs are bringing unforeseen headaches to dairy processors. If dairy farmers would religiously withhold milk from treated cows for a period of 72 hours or six milkings after treatment, the dairy processors' "starter" bacteria would encounter little difficulty in producing acids and certain flavor compounds in cultured buttermilk, fermented milk drinks, and cottage, cheddar and other types of cheese. Very often the affected milk is not held off the market, and small quantities of penicillin, aureomycin or other antibiotics in the milk slow the production of acid or, if present in concentrations of 0.25 μg./ml. or more, prohibit acid production entirely. H. E. Walter and co-workers (*242d*) at the Bureau of Dairy Industry, USDA, Washington, D. C., have studied the problem of employing a dye which could be injected along with the antibiotic into the udders of infected cows. The object in this case was to obtain a dye which would be shed along with the antibiotic and serve as a warning that the milk was not to be included in the regular supply. The specific requirements of such a dye are as follows: It must be non-toxic to mammary tissue, soluble in milk and impart a brilliant color to it; must not be reduced by microorganisms; must not destroy the antibiotic activity of the drug; and must be shed in the milk as long as the antibiotic is shed. The dye, Amaranth 2, was found to meet these requirements adequately for the most part.

DISCUSSION

Although a large number of actinomycetous antibiotic factors have been described and are discussed herein, the list is far from complete. Beyond any doubt, many more factors have been isolated and incompletely studied by the large pharmaceutical and biological firms here and abroad and have been shelved primarily

because of toxicity or other undesirable characteristics. Other new agents demonstrated in screening programs show low spectrum plate activity and are never produced in liquid culture. Occasionally strains showing good spectrum plate activity give little or no antibiotic on subsequent testing in shaken flask cultures. It is possible that some of these factors would be produced by surface culture techniques. Other possible sources, often ignored by those employing spectrum plate techniques, are the strains which exhibit extremely low or non-diffusible activity. There are many examples where a complex is produced, from which the major component is isolated and further characterized, but the minor fractions are not, due to low quantities present and the difficulty of separating one from another.

It is interesting to compare the general chemical nature of the actinomycetous antibiotics with those isolated from higher fungi. Brian (21) points out that nearly all of the latter are lipoid-soluble, only sparingly water-soluble, and that a high proportion are acidic. Only 12 out of 54 such compounds contain nitrogen. In contrast, among the actinomycetous antibiotics, basic substances thus far outnumber acidic compounds by two to one, as exemplified by the fact that only nine compounds out of 50 are lacking in nitrogen. Both large groups of antibiotics are similar in showing no chemical unity as a class. However, a number of antibiotic families have begun to appear, wherein substances within the family exhibit similar antibiotic activity and are also related in a chemical way. Antibiotics in this category include the following: the streptomycin group; actinomycin and actinochrysin; aureomycin and terramycin; aureothricin, thiolutin and thioaurin. Although the chemical relationships are not yet clarified, other possible family groups would include Magnamycin and Ilotycin; streptothricin, streptolin, antibiotic 136, and streptin; the neomycin(s), catenulin, dextromycin and flavomycin, and picromycin and the proactinomycins. The few structural formulae worked out for the actinomycete group represent considerable diversity, and it is doubtful that many additional compounds whose empirical formulae are now known will be analyzed structurally in the immediate future, due partially to their complexity and also to the lack of any probable commercial applications.

Pending the insertion of new antibiotic-producing species into their proper groups in the present classification scheme, one is not

able to draw any conclusions as to the relationships of described species which produce the same or closely related factors. One of the puzzling problems regarding those species which have yielded medically significant antibiotics is their apparent rare occurrence in soils. None of these strains is particularly fastidious with respect to cultivation on artificial media. The studies of Gottlieb and Siminoff (79) and Gregory et al. (80*a*) would seem to indicate that trace amounts of antibiotic are produced by certain antagonistic strains in sterile soil but none is produced in non-sterile soil.

Approximately 115 antibiotics have been covered in this review. A majority of them have been obtained in pure or highly purified form and appear to be well characterized. With the exception of several uncolored bases and grisein, water solubility is relatively low. Without counting possible synonyms and chemical variants, 38 factors exhibit a fairly wide spectrum of activity, 28 are mainly anti Gram-positive, 12 are strictly antifungal, eight have a narrow selective spectrum, five are active mainly against acid-fast strains, and three factors are antiviral.

From a medical standpoint, as emphasized in Raper's excellent review (175), much was accomplished during the " Antibiotic Decade ", 1941–1951. Our need for a less toxic and more effective antibiotic or chemosynthetic against tuberculosis may be partially fulfilled by isoniazid and related compounds. Exhaustive searches for effective antiviral compounds have yet to bear fruit. Although the need is perhaps less pressing, the same is true for the systemic types of fungal infections. The spheres of application of the antibiotics have expanded materially, and other possible applications may develop which now are totally unforeseen.

SUMMARY

Alphabetical classification of the various antibiotics described from actinomycetes with reference to their chemical, physical and biological properties forms a major portion of this review. Other aspects include numerical data on antagonistic actinomycetes isolated from naturally occurring materials, antagonistic properties of species within the four genera considered, and the practical applications of vitamin B_{12}-antibiotic supplements in the feeding of non-ruminant animals.

Excluding possible synonyms and chemical derivatives, antibiotics thus far reported from the genera *Actinomyces, Streptomyces, Nocardia* and *Micromonospora* total 94, the major portion of which

are fairly well characterized. Approximately 91% of these originate from species of *Streptomyces*. The relatively few antibiotics obtained from the genus *Nocardia*, which contains about one-third the number of species classified as *Streptomyces*, is not necessarily a true reflection of the potentialities of this group.

In determining the microbial toxicity of the actinomycetous antibiotics, emphasis has been placed primarily on the growth-inhibiting properties against microorganisms of medical significance. The scope of activity includes compounds which show a very narrow selective action within a single group to those having exceedingly broad spectrums affecting certain organisms within each of the major groups. In addition to antibacterial, antifungal, and antiviral activity, two antibiotics possess insecticidal power.

An arbitrary classification of actinomycetous antibiotics has been devised which separates pigmented substances on the basis of solubilities and non-pigmented substances according to chemical characteristics.

Structural formulae thus far determined (excluding the relationships of the streptomycin series) show no chemical unity as a class and exhibit unique structures not heretofore encountered by organic chemists in naturally occurring materials.

The need for additional criteria for the difficult task of identifying antibiotic-producing actinomycetes has been pointed out.

ACKNOWLEDGMENTS

The author is indebted to Drs. Kenneth B. Raper, Richard W. Jackson, and J. C. Lewis for technical reviews of the manuscript and wishes to thank Drs. R. D. Coghill, H. B. Woodruff, S. A. Waksman, J. D. Dutcher, N. O. Sjolander, A. C. Finlay, E. M. Weber, H. E. Carter, C. P. Hegarty, H. Umezawa, W. Szybalski, N. Komatsu and many other colleagues in universities and pharmaceutical companies, both here and abroad, for their kindness in supplying reprints, papers in press, and personal information.

ADDENDUM

After the final manuscript was submitted to the Editor, the following additional factors were noted by the reviewer up to January 1, 1953.

Fuscomycin is the name assigned to antibiotic 91-6 of Oki and Hata. Other factors briefly discussed in the reference cited below

are flaveolin, and orientomycin. The first two substances are related to the xanthomycins according to Ishida and Miyazaki (J. Antibiotics **5**: 481–487. 1952).

Two new antifungal substances, "moldin" from a strain of *S. phaeochromogenus* and "phaeofacin" from *S. phaeofaciens* n. sp., were reported by Maeda et al. (J. Antibiotics **5**: 465. 1952) and Umezawa et al. (J. Antibiotics **5**: 466. 1952) have crystallized "exfoliatin" ($C_{27}H_{40}O_{16}Cl \cdot H_2O$) from culture liquors of a strain of *S. exfoliatus*.

Hata et al. (J. Antibiotics **5**: 529–534. 1952) have renamed the luteomycin strain *S. tanashiensis* (cited in the review as a variant of *S. aureus*) and crystalline luteomycin hydrochloride is believed to be $C_{23}H_{29}NO_9 \cdot HCl$, in studies reported by Sano (J. Antibiotics **5**: 535–538. 1952).

REFERENCES

1. Adámek, R. et al. Antibiotická plisen *Hyphomyces* antibioticus a její produckt mycelin. Biol. Listy [Prague] **31**: 65–69. 1950.
1*a*. Aiso, K. et al. Flavomycin, an antibiotic produced by *Streptomyces* No. 320. Jour. Antibiotics [Japan] **2**: 87–92. 1949.
1*b*. ————. "Mycelin", a new antifungal substance extracted from the mycelium of a *Streptomyces*. Jour. Antibiotics [Japan] **4**: 217–219. 1952.
2. Ambrose, A. M. Comparative studies on toxicity of a new streptomycin and streptomycin sulfate. Proc. Soc. Exp. Biol. & Med. **76**: 466. 1951.
3. Anchel, M. Acetylenic compounds from fungi. Jour. Am. Chem. Soc. **74**: 1588–1590. 1952.
3*a*. Anderson, H. W. and Gottlieb, D. Plant disease control with antibiotics. Econ. Bot. **6**: 294–308. 1952.
4. Arai, T. Studies of flavomycin. Taxonomic investigations on the strain, production of the antibiotic and application of cup method to the assay. Jour. Antibiotics [Japan] **4**: 215–221. 1951.
4*a*. Ark, P. A. and Oswald, J. W. Antibiotic potentialities of some strains of *Streptomyces scabies*. Phytopath. **41**: 1034. 1951.
5. Arnstein, H. R. V. et al. The inhibition of *Fusarium oxysporum* var. *cubense* by musarin, an antibiotic produced by Meredith's actinomycete. Jour. Gen. Microb. **2**: 111–122. 1948.
5*a*. Asheshov, I. N. et al. Phagolessin A58: A new antibiotic active against bacterial viruses. Antibiotics & Chemotherapy **2**: 366–374. 1952.
6. Baram, P. and Meyer, E. Antifungal substances from a soil actinomycete. Paper presented at the Soc. Ill. Bact. Meeting, Peoria, Illinois. 1951.
7. Bartz, Q. R. Isolation and characterization of Chloromycetin. Jour. Biol. Chem. **172**: 445–450. 1948.
8. ———— et al. Dihydrostreptomycin. Jour. Am. Chem. Soc. **68**: 2163–2166. 1946.
9. ———— et al. Viomycin, a new tuberculostatic antibiotic. Am. Rev. Tuberc. **63**: 4–6. 1951.
10. Benedict, R. G. and Lindenfelser, L. A. [Unpub.]

11. ———— et al. Cinnamycin, an antibiotic from *Streptomyces cinnamoneus*, n. sp. Antibiotics & Chemotherapy. [In press].
11*a*. ———— et al. Morphological and physiological studies on *Streptomyces cinnamoneus*. Antibiotics & Chemotherapy. [In press].
12. ———— *et al.* Studies on *Streptomyces griseocarneus* and the production of hydroxystreptomycin. Jour. Bact. **62**: 487–497. 1951.
13. ———— et al. A new streptomycin. Science **112**: 77–78. 1950.
13*a*. ———— and LINDENFELSER, L. A. Production of soluble pigments by certain strains of *Streptomyces griseus*. Antibiotics & Chemotherapy **1**: 512–517. 1951.
14. BENNETT, R. E. [Personal communication].
15. BERGER, J. et al. Borrelidin, a new antibiotic with anti-borrelia activity and penicillin enhancement properties. Arch. Biochem. **22**: 476–478. 1949.
15*a*. ———— et al. The isolation of three new crystalline antibiotics from *Streptomyces*. Jour. Am. Chem. Soc. **73**: 5295–5298. 1951.
15*b*. BICK, I. R. et al. The isolation and properties of nocardianin. Antibiotics & Chemotherapy **2**: 255–258. 1952.
16. BIELY, J. and MARCH, B. E. The effect of aureomycin and vitamins on the growth rate of chicks. Science **114**: 330–331. 1951.
17. BIRD, H. R. et al. Relation of an unidentified dietary factor to the utilization of vegetable proteins by chickens. Off. Rep. VIII World's Poul. Cong. 1948.
18. BLIGHT, J. C. The story of antibiotics in swine nutrition. Feed Age **1**: 12–15, 50. 1951.
19. BOHONOS, N. et al. A new antibiotic produced by a strain of *Streptomyces lavendulae*. Arch. Biochem. **15**: 215–225. 1947.
19*a*. BOLHOFER, W. A. et al. Thioaurin, a new antibiotic. 122nd Meeting, Am. Chem. Soc., Atlantic City, 1952, p. 12A. [Abs.].
19*b*. BRANION, H. D. and HILL, D. C. The comparative effect of antibiotics on the growth of poults. Poultry Sci. **30**: 793–798. 1951.
20. BRAZHNIKOVA, M. G. The isolation, purification and properties of litmocidin. Jour. Bact. **51**: 655–657. 1946.
20*a*. BREED, R. S. et al. The Bergey Manual of Determinative Bacteriology. Sixth Ed., pp. 892-981. 1948.
21. BRIAN, P. W. Antibiotics produced by fungi. Bot. Rev. **17**: 357–430. 1951.
22. BROCKMANN, H. and BAUER, K. Rhodomycin, ein rotes Antibioticum aus Actinomyceten. Naturwiss. **37**: 492–413. 1950.
23. ———— and GRUBHOFFER, N. Actinomycin C. Naturwiss. **36**: 376–377. 1949.
24. ———— and ————. Zur Kenntnis des Actinomycins C. Naturwiss. **37**: 494–496. 1950.
25. ———— et al. Über das Actinomycin C (Antibiotica aus Actinomyceten. V. Mitteil.) Chem. Ber. **84**(3) : 260–284. 1951.
26. ———— and HENKEL, W. Pikromycin, ein neues Antibiotikum aus Actinomyceten. Naturwiss. **37**: 138–139. 1950.
27. ———— and ————. Pikromycin, ein bitter schmeckendes Antibioticum aus Actinomyceten (Antibiotika aus Actinomyceten. VI. Mitteil). Chem. Ber. **84**: 284–288. 1951.
27*a*. ———— et al. Pikromycin, II. Mitteil (Antibiotika aus Actinomyceten. IX. Mitteil). Chem. Ber. **85**: 426–433. 1952.
28. ———— and PINI, H. Actinorhodin, ein roter Farbstoff aus Actinomyceten. Naturwiss. **34**: 190. 1947.
29. ———— and SCHMIDT-KASTNER, G. Resistomycin, ein neues Antibioticum aus Actinomyceten Naturwiss. **38**: 479–480. 1951.
30. ———— et al. Antibiotica aus Actinomyceten. VII. Rhodomycin, ein rotes Antibioticum. Chem. Ber. **84**: 700–710. 1951.

31. BROSCHARD, R. W. et al. Aureomycin, a new antibiotic. Science **109**: 199–200. 1949.
31*a*. BROWNLEE, G. Antibiotics (with particular reference to mode of action). Ann. Rev. Microbiol. **5**: 197–208. 1951.
32. BRYER, M. S. et al. Treatment of experimental infections with aureomycin. Ann. N. Y. Acad. Sci. **51**: 254–266. 1948.
33. BUCK, M. et al. The anti-*Borrelia* effect of borrelidin. Trans. N. Y. Acad. Sci. II. **11**: 207–210. 1949.
34. BUMPUS, M. et al. The chemistry of antimycin A. 121st Nat. Am. Chem. Soc. Meet., p. 7C, Milwaukee. 1952. [Abs.].
35. BURKHOLDER, P. R. Studies on antibiotic activity of actinomycetes. Jour. Bact. **52**: 503–504. 1946.
36. CARPENTER, L. E. Effect of aureomycin on the growth of weaned pigs. Arch. Biochem. **27**: 469–471. 1950.
37. ———. The effect of antibiotics and vitamin B_{12} on the growth of swine. Arch. Biochem. & Biophys. **32**: 187–191. 1951.
38. CARTER, H. E. et al. Chemistry of streptothricin and related antibiotics. Abstr. of papers, 120th Meet. Am. Chem. Soc., New York, p. 3L. 1951.
39. ——— et al. Chloromycetin and streptothricin. Science **107**: 113. 1948.
39*a*. ——— et al. Structure of the diaminohexanoic acid from streptothricin. Jour. Am. Chem. Soc. **74**: 3704. 1952.
40. CELMER, W. D. and SOLOMONS, I. A. Mycomycin. Isolation, crystallization, and chemical characterization. 121st Meet. Am. Chem. Soc., Milwaukee, p. 17J. 1952. [Abs.]
41. ——— and ———. Mycomycin. II. The structure of isomycomycin, an alkali-isomerization product of mycomycin. Jour. Am. Chem. Soc. **74**: 3838–3842. 1952.
42. ——— and ———. Mycomycin. I. Isolation, crystallization, and chemical characterization. Jour. Am. Chem. Soc. **74**: 2245–2248. 1952.
43. ——— and ———. The structure of the antibiotic mycomycin. Jour. Am. Chem. Soc. **74**: 1870–1871. 1952.
43*a*. ——— et al. Characterization of the antibiotic thiolutin and its relationship with aureothricin. Jour. Am. Chem. Soc. **74**: 6304. 1952.
44. CERCOS, A. P. Actinomycelina, nuevo antibiotico producido por un *Streptomyces*. Pub. Tecnica No. 16, Inst. Fitotecnia: 147–156. 1948.
45. ——— and RODRIGUEZ, H. T. Actinomycetes antibioticos de la Argentina. Rev. Argentina Agron. **18**: 65–73. 1951.
45*a*. CHARNEY, J. et al. Pleocidin, a new antibiotic related to streptothricin. Antibiotics & Chemotherapy **2**: 307–310. 1952.
45*b*. ——— et al. Rhodocidin; a new antibiotic. 122nd Meeting, Am. Chem. Soc., Atlantic City, p. 13A. 1952. [Abs.]
46. CLARK. R. K., JR. and SCHENCK, J. R. III. The synthesis of actithiazic acid, derivatives, and homologs. Arch. Biochem. & Biophysics. [In press].
47. CONTROULIS, J. et al. Chloramphenicol (Chloromycetin). V. Synthesis. Jour. Am. Chem. Soc. **71**: 2463–2468. 1949.
47*a*. CRAM, D. J. [Personal communication].
48. DALGLEISH, C. E. and TODD, A. R. Actinomycin. Nature **164**: 830. 1949.
49. ——— and ———. Actinomycin B. Abst. Commun., First Int. Cong. of Biochem., Cambridge, England, pp. 246–247. August 19-25, 1949.
50. ——— et al. Actinomycin. Part I. Amino acid content. Jour. Chem. Soc. [London]: 2946–2952. 1950.

51. DARPOUX, H. and FAIVRE-AMIOT, H. Sur un actinomycète doué de propriétés bactériolitiques remarquables. Compt. Rend. [Paris] **226:** 1146–1148. 1948.
52. DAVISSON, J. W. et al. Rimocidin, a new antibiotic. Antibiotics & Chemotherapy **1:** 289. 1951.
52*a*. ———— et al. Catenulin, a new antibiotic. Antibiotics & Chemotherapy **2:** 460–461. 1952.
52*b*. DORNBUSH, A. C. and PELCAK, E. J. The determination of aureomycin in serum and other body fluids. Ann. N. Y. Acad. Sci. **51:** 218–220. 1948.
53. DUGGAR, B. M. et al. Aureomycin, a new antibiotic. Ann. N. Y. Acad. Sci. **51:** 177–331. 1948.
53*a*. ————. Aureomycin and preparation of the same. U. S. Patent No. 2,482,055. September 13, 1949.
53*b*. DUNITZ, J. D. and LEONARD, J. E. X-ray analysis of some antibiotic substances. Jour. Am. Chem. Soc. **72:** 4276–4277. 1950.
54. ———— and ROBERTSON, J. H. Relationship between aureomycin and terramycin. Jour. Am. Chem. Soc. **74:** 1108. 1952.
55. DUNSHEE, B. R. et al. Isolation and properties of antimycin A. Jour. Am. Chem. Soc. **71:** 2436–2437. 1949.
56. DUTCHER, J. D. and DONIN, M. M. The identity of neomycin A, neamine and methanolysis product of neomycin B and C. Jour. Am. Chem. Soc. **74:** 3420–3422. 1952.
57. ————. [Personal communication].
58. ———— et al. Neomycins B and C and some of their degradation products. Jour. Am. Chem. Soc. **73:** 1384–1385. 1951.
59. Editorial (no authors). Colonial research on antibiotics. Manufacturing Chemist **22:** 47. 1951.
60. EHRLICH, J. et al. Chloromycetin, a new antibiotic from a soil actinomycete. Science **106:** 417. 1947.
61. ———— et al. *Streptomyces venezuelae* n. sp., the source of chloromycetin. Jour. Bact. **56:** 467–477. 1948.
62. EMERSON, R. L. et al. Studies on the production of antibiotics by actinomycetes and molds. Jour. Bact. **52:** 357-366. 1946.
63. EMMART, E. W. A new tuberculostatic antibiotic from a species of *Nocardia*. Am. Rev. Tuberc. **56:** 316-333. 1947.
64. ———— et al. The tuberculostatic action of *Nocardia* extracts in white mice. Jour. Bact. **57:** 509–514. 1949.
65. FAINSHMIDT, O. I. and KORENIAKO, A. I. Preparation of concentrates of the antimicrobial substance produced by *Actinomyces violaceus*. Biokhimiya **9:** 147–153. 1944.
66. FINLAY, A. C. et al. Netropsin, a new antibiotic produced by a *Streptomyces*. Jour. Am. Chem. Soc. **73:** 341–343. 1951.
67. ———— and SOBIN, B. A. Netropsin and process for its production. U. S. Patent No. 2,586,762. February 19, 1952.
68. ———— et al. Terramycin, a new antibiotic. Science **111:** 85. 1950.
69. ———— et al. Viomycin, a new antibiotic active against mycobacteria. Am. Rev. Tuberc. **63:** 1–3. 1951.
70. FISHER, W. P. et al. An actinomycin from a species of the genus *Micromonospora*. Antibiotics & Chemotherapy **1:** 571–572. 1951.
70*a*. FISHER, W. P. and CHARNEY, J. A turbidimetric assay for pleocidin. Antibiotics & Chemotherapy **2:** 311–314. 1952.
71. FRIED, J. and TITUS, E. Streptomycin B, an antibiotically active constituent of streptomycin concentrates. Jour. Biol. Chem. **168:** 391–392. 1947.
72. ———— and ————. Streptomycin. VIII. Isolation of mannosidostreptomycin (streptomycin B). Jour. Am. Chem. Soc. **70:** 3615–3618. 1948.

73. ———— and WINTERSTEINER, O. Crystalline reineckates of streptothricin and streptomycin. Science **101**: 613–615. 1945.
74. GARDNER, A. D. and CHAIN, E. Proactinomycin, a bacteriostatic produced by a species of *Proactinomyces*. Brit. Jour. Exp. Path. **23**: 123–127. 1942.
75. GARSON, W. and WAKSMAN, S. A. Strain specificity and production of antibiotic substances. VIII. Production of a grisein-like antibiotic by a strain of *S. griseus*. Proc. Nat. Acad. Sci. **34**: 232–239. 1948.
75*a*. GOTH, A. and ROBINSON, F. J. Pharmacological studies on Actidione. Jour. Clin. Invest. **28**: 1044. 1949.
76. GAUSE, G. F. A new antibiotic substance produced by *Proactinomyces cyaneus*. Jour. Bact. **51**: 649–653. 1946.
77. GOTTLIEB, D. et al. Some properties of an antibiotic obtained from a species of *Streptomyces*. Jour. Bact. **55**: 409–417. 1948.
78. ———— et al. Endomycin, a new antibiotic. Phytopath. **41**: 393–400. 1951.
79. ———— and SIMINOFF, P. Production and activity of Chloromycetin in soils. Bact. Proc.: 16–17. 1951.
80. GORET, M. et al. Extraction du mycélium d'*Actinomyces griseus*, d'un antibiotique différent de la streptomycine et de la griséine. Compte. Rend. **229**: 677–682. 1949.
80*a*. GREGORY, K. F. et al. Production and stability in soil of some antibiotics active against fungi. Bact. Proc.: 18. 1951.
81. GREIFF, D. and PINKERTON, H. Rickettsiostasis in fertile eggs from use of antibiotic residues in poultry feeds. Proc. Soc. Exp. Biol. & Med. **78**: 690–692. 1951.
82. GROUPÉ, V. et al. Ehrlichin, a new antibiotic with specific antiviral properties. Bact. Proc., 51st Meet. S.A.B.: 29. 1951.
82*a*. ———— et al. Antiviral properties of ehrlichin, an antibiotic produced by *Streptomyces lavendulae*. Jour. Immunol. **67**: 471–482. 1951.
83. GRUNBERG, E. et al. Enhancement of penicillin activity by borrelidin. Trans. N. Y. Acad. Sci. II. **11**: 210–214. 1949.
84. GRUNDY, W. E. et al. A note on a new antibiotic. Arch. Biochem. **28**: 150–152. 1950.
85. ———— et al. A study of *Streptomyces* NA 232-MI and hydroxystreptomycin. Antibiotics & Chemotherapy **1**: 309–317. 1951.
86. ———— et al. Actithiazic acid. I. Microbiological studies. Antibiotics & Chemotherapy **2**: 399–408. 1952.
87. HAMRE, D. M. et al. Some characteristics of the neomycins. Antibiotics & Chemotherapy **2**: 135–141. 1952.
88. HARNED, R. L. et al. Nigericin, a new crystalline antibiotic from an unidentified *Streptomyces*. Antibiotics & Chemotherapy **1**: 594–596. 1951.
89. HASKELL, T. H. et al. The chemistry of viomycin. Jour. Am. Chem. Soc. **74**: 599–602. 1952.
90. HATA, T. et al. Isolation of a new antibiotic substance, "Luteomycin", from a strain of *Streptomyces*. Kitasato Arch. Exp. Med. **32**: 229–242. 1949.
91. ———— et al. Isolation of a new antibiotic substance, "luteomycin". Jour. Antiobiotics [Japan] **3**: 313–325. 1950.
92. HATSUTA, Y. Studies on the antibiotic litmus-like pigment produced by *S. coelicolor*. II. On coelicolorin. Jour. Antibiotics [Japan] **2**: 276–277. 1949.
93. HAZEN, E. L. and BROWN, R. Fungicidin, an antibiotic produced by a soil actinomycete. Proc. Soc. Exp. Biol. & Med. **76**: 93–97. 1951.
94. HEATLEY, N. G. [Unpub., cited from Marston (146)].

95. HENRY, A. W. et al. Control of covered smut of wheat by rapid seed treatment with an antibiotic. Science **115**: 90–91. 1952.
96. HEUSER, L. J. et al. The crystalline trihydrochlorides of streptomycin and mannosidostreptomycin. Jour. Am. Chem. Soc. **70**: 2833–2834. 1948.
97. HICKEY, R. J. and HIDY, P. H. Crystalline fradicin. Science **113**: 261–262. 1951.
97*a*. ———— et al. Ascosin, an antifungal antibiotic produced by a streptomycete. Antibiotics & Chemotherapy **2**: 472–483. 1952.
98. HIDY, P. H. and HICKEY, R. J. The isolation of crystalline fradicin from fermentation media. Arch. Biochem. & Biophys. **34**: 67–71. 1951.
99. HIRATA, Y. and NAKANISHI, K. Substance from *Actinomyces flavus*. Jour. Antibiotics [Japan] **2**: 180–182. 1948.
100. ———— and ————. Actinomycin J_3, a byproduct from a strain of *Actinomyces*. Agr. Chem. Soc. Japan, Bull. **22**: 121–127. 1949.
101. HOBBY, G. L. Current status of the new antituberculous agents. Minutes of the Eighth Streptomycin Conference, Vet. Adm., Army, Navy, 295–298. 1949.
101*a*. HOCHSTEIN, F. A. et al. Terramycin. V. Structure of terrinolide. An acid degradation product of terramycin. Jour. Am. Chem. Soc. **74**: 3706–3707. 1952.
101*b*. ———— et al. Terramycin. VI. The structure of α- and β-apoterramycin, acid rearrangement products of terramycin. Jour. Am. Chem. Soc. **74**: 3707-3708. 1952.
101*c*. ———— et al. Terramycin. VII. The structure of terramycin. Jour. Am. Chem. Soc. **74**: 3708–3709. 1952.
102. HOSOYA, S. et al. A new antibiotic, reticulin, produced by *Streptomyces reticuli*. Japan Jour. Exp. Med. **20**: 327–335. 1949.
103. ———— et al. Xanthomycin-like substance produced by a species of *Streptomyces*, H-1159 strain. Jour. Antibiotics [Japan] **4**: 467–469. 1951.
104. ———— et al. On the antibiotic substances produced by *Streptomyces* H-702. Jour. Antibiotics [Japan] **4**: 317–320. 1951.
105. ———— et al. Studies on the several antibiotic substances produced by a *Streptomyces* (H-277 strain). Jour. Antibiotics [Japan] **4**: 314–316. 1951.
105*a*. ———— et al. Rotaventin, an antifungal agent isolated from mycelium of *Streptomyces reticuli*. Jap. Jour. Exp. Med. **21**: 279–283. 1951.
105*b*. ———— et al. An antifungal agent isolated from mycelium of *Streptomyces griseocarneus*. Jap. Jour. Exp. Med. **22**: 23–25. 1952.
105*c*. ———— et al. Studies on the antibiotic substance produced by *Streptomyces* H-1205. Jour. Antibiotics [Japan] **5**: 245–247. 1952.
105*d*. ———— et al. Comparison of reticulin and hydroxystreptomycin. [In press].
105*e*. ———— et al. Trichomycin, a new antibiotic produced by *Streptomyces hachijoensis*, with trichomonadicidal and antifungal activity. [In press].
106. HUTCHINGS, B. L. et al. Degradation of aureomycin. V. Aureomycinic acid. Jour. Am. Chem. Soc. **74**: 4980. 1952.
106*a*. HUTCHINSON, D. et al. Production, isolation, and antimicrobial, notably antituberculous properties of streptothricin VI. Arch. Biochem. **22**: 16–30. 1949.
107. HWANG, K. Actithiazic acid. IV. Pharmacological studies. Antibiotics & Chemotherapy **2**: 453–459. 1952.
108. IKEDA, Y. et al. Studies on the antifungal antibiotics from actinomycetes. Jour. Antibiotics [Japan] **3**: (11): 726–729. 1950.

109. ISENBERG, H. D. et al. Oxidation of ammonia by *Streptomyces nitrificans*, a new soil actinomycete. Bact. Proc.: 152. 1952. [Abs.]
110. ISHIDA, K. Studies on the antibiotic substances from *Actinomyces*. IX. A new basic antibiotic produced by a strain of *Streptomyces roseochromogenus*, No. 36. Jour. Antibiotics [Japan] **3**: (13): 845–853. 1950.
111. JAMPOLSKY, L. M. and GOLDBERG, M. W. Isolation of crystalline borrelidin and of borrelidin-like concentrates from crude penicillin. Jour. Clin. Invest. **28**: 1046. 1949.
112. JENKINS, D. E. The present status of mycomycin, a progress note. Minutes of the Tenth Streptomycin Conference, Vet. Adm., Army, Navy, 286–287. 1951.
113. JEZESKI, J. J. Dairy plants face antibiotic problem. Minn. Farm & Home Sci. **9**: 16. 1952.
114. JOHNSON, E. A. and BURDON, K. L. Mycomycin, a new antibiotic produced by a mold-like actinomycete active against the bacilli of human tuberculosis. Jour. Bact. **54**: 281. 1947.
115. JOHNSTONE, D. B., and WAKSMAN, S. A. The production of streptomycin by *Streptomyces bikiniensis*. Jour. Bact. **55**: 317–326. 1948.
116. JUNOWICZ-KOCHOLATY, R. and KOCHOLATY, W. Lavendulin and actinorubin. II. (lavendulin and actinorubin) Two antibiotics produced by two strains of actinomycetes. Purification and isolation. Jour. Biol. Chem. **168**: 757–764. 1947.
117. ———— et al. Sulfactin, a new antibiotic produced by a soil *Actinomyces*. Jour. Biol. Chem. **168**: 765–769. 1947.
117*a*. KAREL, L. and ROACH, E. S. A dictionary of antibiosis. Columbia University Press, New York. 1951.
118. KELNER, A. and MORTON, H. E. An antibiotic produced by an actinomycete specifically inhibiting species of *Mycobacterium*. Proc. Soc. Exp. Biol. & Med. **63**: 227–230. 1946.
119. ———— and ————. Two antibiotics (lavendulin and actinorubin) produced by *Actinomyces*. I. Isolation and characteristics of the organisms. Jour. Bact. **53**: 695–704. 1947.
120. ———— et al. Two antibiotics produced by *Actinomyces* isolated from soil. Jour. Bact. **51**: 591. 1946.
120*a*. KIDO, G. S. and SPYHALSKI, E. An antibiotic with insecticidal and miticidal properties. Science **112**: 172–173. 1950.
121. KIRBY, W. M. M. Antibiotics. Ann. Rev. Microbiol. **6**: 387–410. 1952.
121*a*. KOBAYASHI, S. et al. Studies on the antibiotic substances from *Actinomyces*. 4th Report. Isolation of streptomycin from *Str. olivaceus*. Tohoku Jour. Exp. Med. **55**: 273–282. 1952.
121*b*. KOCHI, M. et al. Antibiotic producing properties of *Streptomyces* 3560, a member of the *S. flavus* group. Proc. Nat. Acad. Sci. **38**: 383–391. 1952.
122. KOCHOLATY, W. et al. Actinomycin A produced by a soil actinomyces different from *A. antibioticus*. Arch. Biochem. **17**: 191–193. 1948.
122*a*. KOMATSU, N. [Personal communication].
123. KORNFELD, E. C. et al. The structure and chemistry of Acti-dione, an antibiotic from *Streptomyces griseus*. Jour. Am. Chem. Soc. **71**: 150–159. 1949.
124. KRASSILNIKOV, N. A. and KORENIAKO, A. I. Bactericidal substance of actinomycetes. Mikrobiologia, Moskva **8**: 673–685. 1939.
124*a*. ———— and ————. Mycetin and its bactericidal properties. Mikrobiologiya (U.S.S.R.) **14**: 80–84. 1945.
125. KRISS, A. E. The lysozyme of actinomycetes. Mikrobiologia, Moskva **9**: 32–38. 1940.
126. KUEHL, F. A., JR. et al. Isolation and some chemical properties of grisein. Jour. Am. Chem. Soc. **73**: 1770–1773. 1951.

126*a*. KUHN, R. and DURY, K. Alkalischer Abbau von Aureomycin und Terramycin. Chem. Ber. **84**: 563–565. 1951.
127. KUPFERBERG, A. B. et al. The production of streptocin by different strains of *Streptomyces griseus*. Jour. Bact. **59**: 523–526. 1950.
128. KUROSAWA, H. Mycological characters of antagonistic *Streptomyces*. I. On the correlation between Pridham's classification method and antibiotic characters. Jour. Antibiotics [Japan] **4**: 183–193. 1951.
129. KUROYA, M. et al. Studies on the antibiotic substances from *Actinomyces*. III. Jour. Antibiotics **2** (B): 37–50. 1949.
130. ———— et al. Studies on antibiotic substances from *Actinomyces*. VI. On the properties of several streptothricin-like substances. Jour. Antibiotics [Japan] **3**: 223–228. 1950.
130*a*. ———— et al. Studies on the antibiotic substances from *Actinomyces*. 1st Report. On the isolation and classification of antibiotic-producing Actinomycetaceae. Tohoku Jour. Exp. Med. **54**: 371–383. 1951.
131. KUSHNICK, T. et al. Variants of *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* requiring streptomycin. Science **106**: 587-588. 1947.
132. LANDERKIN, G. B. and LOCHHEAD, A. G. [Unpub., cited from reference 133].
133. ———— et al. A study of the antibiotic activity of actinomycetes from soils of Northern Canada. Canad. Jour. Res., C. **28**: 690–698. 1950.
134. LARSON, L. M. et al. Production, isolation, and components of the antibiotic streptolin. 121st Meeting Am. Chem. Soc., Milwaukee, p. 8C. 1952. [Abs.]
135. LEACH, B. E. et al. The isolation and characterization of neomycin. Jour. Am. Chem. Soc. **73**: 2797–2800. 1951.
136. ———— and TEETERS, C. M. Neamine, an antibacterial degradation product of neomycin. Jour. Am. Chem. Soc. **73**: 2794–2797. 1951.
136*a*. ————and ————. The identity of neamine and neomycin A. Jour. Am. Chem. Soc. **74**: 3187–3188. 1952.
137. LEBEN, C. and KEITT, G. W. The effect of an antibiotic substance on apple leaf infection by *Venturia inaequalis*. Phytopath. **37**: 14. 1947.
138. ———— and ————. An antibiotic substance active against certain phytopathogens. Phytopath. **38**: 899–906. 1948.
139. ———— et al. An antibiotic that inhibits certain phytopathogens. Phytopath. **41**: 23–24. 1951.
139*a*. ———— et al. Helixin, an antibiotic active against certain fungi and bacteria. Mycologia **44**: 159–169. 1952.
140. LEHR, H. and BERGER, J. The isolation of a crystalline actinomycin-like antibiotic. Arch. Biochem, **23**: 503–505. 1949.
141. LEUCKE, R. W. et al. Effects of vitamin B_{12}, animal protein factor, and streptomycin on the growth of young pigs. Arch. Biochem. **26**: 326–327. 1950.
142. ———— et al. The growth-promoting effect on pigs of a surface active agent. Mich. Quart. Bull. **34**: 331–332. 1952.
142*a*. LEWIS, J. C. [Personal communication.]
142*b*. ———— and SNELL, M. S. The amino acid composition of subtilin. Jour. Am. Chem. Soc. **73**: 4812–4816. 1951.
142*c*. LINDENBEIN, W. Über einige chemisch interresante Aktinomycetenstämme und ihre Klassifizierung. Archiv Microbiologie **17**: 361–383. 1952.
143. LOUGHLIN, E. H. et al. The treatment of enterobiasis with terramycin base. Antibiotics & Chemotherapy **1**: 588–593. 1951.
143*a*. LUMB, M. Antibiotics. Rpts. Prog. Applied Chem. **36**: 493–513. 1951.

144. LUTHER, H. G. Synthetic milk for animal production. 17th Annual Conf., Nat. Farm Chemurgic Coun., St. Louis. 1952.
144a. MACHLOWITZ, R. A. et al. Cardicin, an antibiotic having antimicrobial and antiviral properties. 122nd Meeting Am. Chem. Soc., Atlantic City, p. 13A. 1952. [Abs.]
145. MAEDA, K. Chemical studies on antibiotic substances. I. Purification of aureothricin and its molecular formula. Jap. Med. Jour. **2**: 85–88. 1949.
145a. ————. Chemical studies on antibiotic substances. II. On streptothricin BI and BII. Jour. Antibiotics [Japan] **5**: 343–347. 1952.
146. MARSTON, R. Q. The isolation of antibiotics produced by *Proactinomyces* (*Nocardia*) *gardneri*. Brit. Jour. Exp. Path. **30**: 398–407. 1949.
147. ———— and FLOREY, H. W. The antibacterial and pharmacological properties of the proactinomycins A, B, and C. Brit. Jour. Exp. Path. **30**: 407–418. 1949.
148. MATTERSON, L. D. and SINGSEN, E. P. A comparison of several antibiotics as growth stimulants in practical chick starting rations. Conn. Agr. Exp. Sta. [Storrs], Bull. 275: 1–20. 1951.
149. MAYER, R. L. et al. Antibiotics from *Actinomyces vinaceus*. Microbiological studies. XII. Int. Cong. Pure & Appl. Chem.: 283–284. 1951. [Abs.].
150. MCGINNIS, J. et al. Response of chicks and turkey poults to vitamin B_{12} supplements produced by fermentation with different organisms. 116th Meet. Am. Chem. Soc.: 42A. 1949.
150a. MCGUIRE, J. M. et al. "Ilotycin", a new antibiotic. Antibiotics & Chemotherapy **2**: 281–283. 1952.
150b. MCLAMORE, W. M. et al. The structure and synthesis of a new thiazolidone antibiotic. Jour. Am. Chem. Soc. **74**: 2946–2947. 1952.
151. MCVAY, L. V. et al. A preliminary report of the successful treatment of amebiasis with aureomycin. Science **109**: 590–591. 1949.
152. MEREDITH, C. H. The antagonism of soil organisms to *Fusarium oxysporum cubense*. Phytopath. **34**: 426–429. 1944.
153. ———— and SEMENIUK, G. The antagonism of some species of actinomycetes in relation to the soil-inhibiting plant pathogens. Iowa Agr. Exp. Sta., Rep. Agr. Res., Part I, 175. 1947.
154. MILLER, C. P. and BONHOFF, M. The development of streptomycin-resistant variants of *Meningococcus*. Science **105**: 620. 1947.
155. MOLD, J. D. and BARTZ, Q. R. An antibiotic related to the xanthomycins. Jour. Am. Chem. Soc. **72**: 1847–1849. 1950.
156. MOLITOR, H. The pharmacology of streptothricin and streptomycin. Ann. N. Y. Acad. Sci. **48**: 101–118. 1946.
157. MORTON, H. E. Sulfactin. Bacterial spectrum, toxicity, and therapeutic studies. Proc. Soc. Exp. Biol. & Med. **66**: 345–348. 1947.
158. ————. Two antibiotics (lavendulin and actinorubin) produced by two strains of *Actinomyces*. III. Toxicity and therapeutic studies. Proc. Soc. Exp. Biol. & Med. **64**: 327–331. 1947.
159. MOORE, P. R. et al. The use of sulfasuxidine, streptothricin, and streptomycin in nutritional studies with the chick. Jour. Biol. Chem. **165**: 437–441. 1946.
160. NAKHIMOVSKAIA, M. I. Antagonism between actinomycetes and soil bacteria. Mikrobiologia, Moskva. **6**: 131–157. 1937.
161. NORRIS, L. C. Newer nutritional developments of importance to feed manufacturers. Proc. VII Distiller's Feed Conference, Cincinnati, pp. 72–86. 1952.
162. OGATA, K. Dextromycin, a new antibiotic by *Actinomyces*. Jour. Antibiotics [Japan] **3**: 440–444. 1950.

163. OKI, N. and HATA, T. Studies on the substance produced by *Actinomyces* which inhibits the homologous strain. Jour. Antibiotics [Japan] **5:** 16–21. 1952.
164. OTT, W. H. et al. Activity of crystalline vitamin B_{12} for chick growth. Jour. Biol. Chem. **174:** 1047–1048. 1948.
164*a*. OUCHI, N. Studies on the antibiotic substances from *Actinomyces*. 7th Report. On the isolation of special anti-phlei factor from an *Actinomyces* in a crystalline form. Tohoku Jour. Exp. Med. **55:** 355–365. 1952.
165. PASTERNACK, R. et al. Alkaline degradation of terramycin. Abstr. of papers. 12th Int. Cong. Pure & Appl. Chem.: 281–282. 1951.
166. ———— et al. Degradation of terramycin. Jour. Am. Chem. Soc. **71:** 2400. 1951.
167. PECK, R. L. et al. *Streptomyces* antibiotics. XXIII. Isolation of neomycin A. Jour. Am. Chem. Soc. **71:** 2590–2591. 1949.
168. ———— et al. Dihydrostreptomycin. Jour. Am. Chem. Soc. **68:** 1390–1391. 1946.
168*a*. ———— and LYONS, J. E. Biochemistry of antibiotics. Ann. Rev. Biochem. **20:** 367–414. 1951.
169. ———— et al. *Streptomyces* antibiotics. VI. Isolation of streptothricin. Jour. Am. Chem. Soc. **68:** 772–776. 1946.
169*a*. PEPINSKY, R. and WATANABE, T. Isomorphism of terramycin and aureomycin hydrochloride. Science **115:** 541. 1952.
169*b*. PEPPLER, H. J. et al. The effect of feeding crude polymyxin D on the intestinal bacteria of chickens. Poul. Sci. **29:** 520–526. 1950.
170. PERLMAN, D. and LANGLYKKE, A. F. The occurrence of mannosidostreptomycinase. Jour. Am. Chem. Soc. **70:** 3968. 1948.
171. PETERSON, D. H. et al. Evidence for the presence of streptothricin in streptolin culture filtrates. Jour. Am. Chem. Soc. **69:** 3145. 1947.
171*a*. PETERSON, W. H. [Personal communication].
171*b*. PORTER, J. N. et al. Achromycin: a new antibiotic having trypanocidal properties. Antibiotics & Chemotherapy **2:** 409–410. 1952.
171*c*. POWELL, H. M. et al. Antimicrobial spectrum of erythromycin. Antibiotics & Chemotherapy. [In press].
172. PRIDHAM, T. G. and GOTTLIEB, D. The utilization of carbon compounds by some Actinomycetales as an aid for species determination. Jour. Bact. **56:** 107–114. 1948.
173. RAKE, G. et al. On some biological characteristics of streptomycin B. Proc. Soc. Exp. Biol. & Med. **65:** 107–112. 1947.
174. RAO, K. V. and PETERSON, W. H. Further studies on the chemical nature of the xanthomycins. Abstracts of papers, 121st Meeting Am. Chem. Soc., Milwaukee, pp. 8C–9C. 1952.
175. RAPER, K. B. A decade of antibiotics. Mycologia **44:** 1–61. 1952.
175*a*. RAUBITSCHECK, F. et al. Production of an antifungal agent of the fungicidin type by *Streptomyces aureus*. Antibiotics & Chemotherapy **2:** 179–183. 1952.
176. REBSTOCK, M. C. et al. Chloramphenicol (Chloromycetin). IV. Chemical studies. Jour. Am. Chem. Soc. **71:** 2458–2462. 1949.
177. REGNA, P. P. and MURPHY, F. X. The isolation of neomycin B. Jour. Am. Chem. Soc. **72:** 1045–1046. 1950.
178. ———— and SOLOMONS, I. A. The chemical and physical properties of terramycin. Ann. N. Y. Acad. Sci. **53:** 229–237. 1950.
179. ———— et al. The stability of streptomycin. Jour. Biol. Chem. **165:** 631–638. 1946.
180. REILLY, H. C. The production and isolation of an antibiotic from a soil actinomycete. Abst. of papers. Bact. Proc.: 26. 1952.
180*a*. A colloquium, "Studies on the growth effect of antibiotics in germfree animals", Lobund Inst., Univ. Notre Dame, June 4, 1952:

LUCKEY, T. D. Effect of feeding antibiotics upon the growth rate of germ-free birds.
REYNIERS, J. A. A critical analysis of the mode of action of antibiotics on producing growth stimulation in higher animals and a suggested experimental approach.
GORDON, H. A. A morphological and biochemical approach.

181. REYNOLDS, D. M. et al. Grisein, a new antibiotic produced by a strain of *Streptomyces griseus*. Proc. Soc. Exp. Biol. & Med. **64**: 50–54. 1947.
182. ——— and WAKSMAN, S. A. Grisein, an antibiotic produced by certain strains of *Streptomyces griseus*. Jour. Bact. **55**: 739–752. 1948.
183. RICKES, E. L. et al. Comparative data on vitamin B_{12} from liver and a new source, *Streptomyces griseus*. Science **108**: 634–635. 1948.
184. RIVETT, R. W. and PETERSON, W. H. Streptolin, a new antibiotic from a species of *Streptomyces*. Jour. Am. Chem. Soc. **69**: 3006–3009. 1947.
185. ROBINSON, H. J. et al. Pharmacological studies on streptothricin. Jour. Pharmacol. & Exp. Therap. **86**: 22–36. 1946.
186. ROUATT, J. W. et al. Distribution of antagonistic properties among actinomycetes isolated from different soils. Antibiotics & Chemotherapy **1**: 185–192. 1951.
187. ROUTIEN, J. B. and FINLAY, A. C. Problems in the search for microorganisms producing antibiotics. Bact. Rev. **16**: 51–67. 1952.
187*a*. ——— and HOFMANN, A. *Streptomyces californicus* productor de viomicina. Arch. Med. Internacional y Antibioticos y Quimi. **1**: (8–9) : 387–389. 1951.
187*b*. RUCKER, R. B. A streptomycete pathogenic to fish. Jour. Bact. **58**: 659–664. 1949.
188. SCHATZ, A. et al. Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria. Proc. Soc. Exp. Biol. & Med. **55**: 66–69. 1944.
189. ——— and WAKSMAN, S. A. Effect of streptomycin and other antibiotic substances upon *Mycobacterium tuberculosis* and related organisms. Proc. Soc. Exp. Biol. & Med. **57**: 244–248. 1944.
189*a*. SCHNEIDER, H. G. et al. Separation and determination of the antimycins. Arch. Biochem. & Biophysics **37**: 147–157. 1952.
190. SCHENCK, J. R. and DE ROSE, A. F. Actithiazic acid. II. Isolation and characterization. Arch. Biochem. & Biophysics. [In press].
191. SCHÖNE, R. An antibiotic which inhibits *Corynebacterium diphtheriae* produced by the S form of *Streptomyces thermophilus*. Antibiotics & Chemotherapy **1**: 176–180. 1951.
191*a*. SCHWARTZ, J. A. et al. Studies of *Coccidioides immitis* with certain antifungal agents. Trans. 11th Conference on the Chemotherapy of Tuberculosis. Veterans Administration: Army: Navy, pp. 86–93. 1952.
191*b*. SENECA, H. et al. Bactericidal, protozoicidal and fungicidal properties of thiolutin. Antibiotics & Chemotherapy **2**: 357–360. 1952.
192. SHOCKMAN, G. and WAKSMAN, S. A. Rhodomycin. An antibiotic produced by a red-pigmented mutant of *Streptomyces griseus*. Antibiotics & Chemotherapy **1**: 68–75. 1951.
193. SMISSMAN, E. E. et al. Chemical studies of the antibiotic streptolin. Abstracts of papers. 121st Meeting Am. Chem. Soc., Milwaukee, p. 8C. 1952.
194. SMITH, N. R. et al. Aerobic mesophilic sporeforming bacteria. U. S. Dept. Agr. Misc. Pub. 559, 112 pages. 1946.
195. SMITH, R. M. et al. Chloromycetin, biological studies. Jour. Bact. **55**: 425–448. 1948.

195*a*. SOBIN, B. A. A new *Streptomyces* antibiotic. Jour. Am. Chem. Soc. **74**: 2947–2948. 1952.
195*b*. STEPHENS, C. R. et al. Terramycin. VIII. Structure of aureomycin and terramycin. Jour. Am. Chem. Soc. **74**: 4976–4977. 1952.
196. STODOLA, F. H. et al. Hydroxystreptomycin, a new antibiotic from *Streptomyces griseocarneus*. Jour. Am. Chem. Soc. **73**: 2290–2293. 1951.
197. STOKSTAD, E. L. R. et al. The multiple nature of the animal protein factor. Jour. Biol. Chem. **180**: 647–654. 1949.
198. ———— et al. Further observations on the animal protein factor. Proc. Soc. Exp. Biol. & Med. **73**: 523–528. 1950.
199. STOLL, A. et al. Nocardamin, ein neues Antibioticum aus einer *Nocardia*-Art. Schweiz Zeit. Path. u. Bakt. **14**: 225–233. 1951.
200. ———— et al. Beiträge zur Konstitutions Aufklärung des Nocardamins. Helv. Chim. Acta **34**: 862–873. 1951.
201. SUHREN, O. Erste klinische Erfahrungen mit Pikromycin, einem neuen Antibioticum aus Actinomyceten, bei der Behandlung von Pyodermien. Medizinische Klinik, No. 25/26, pages 722–723. 1951.
202. SWART, E. A. Use of countercurrent distribution for characterization of *Streptomyces* antibiotics. Jour. Am. Chem. Soc. **71**: 2942–2944. 1949.
203. ———— et al. Neomycin, recovery and purification. Arch. Biochem. **24**: 92–103. 1949.
204. ———— et al. The identity of the neomycin complex, as measured by countercurrent distribution and microbiological analyses. Jour. Am. Chem. Soc. **73**: 3253–3255. 1951.
205. ———— et al. Fradicin, an antifungal agent produced by *Streptomyces fradiae*. Proc. Soc. Exp. Biol. & Med. **73**: 376–378. 1950.
205*a*. SZYBALSKI, W. [Personal communication].
205*b*. ———— and BRYSON, V. Genetic studies on microbial cross resistance to toxic agents. I. Cross resistance of *Escherichia coli* to fifteen antibiotics. Jour. Bact. **64**: 489–499. 1952.
205*c*. TAIRA, T. and FUJII, S. New antibiotics produced by *Micromonospara* sp., Microcin A and B. Jour. Antibiotics [Japan] **4**: 185–187. 1952.
205*d*. TAKAHASHI, I. Studies on the antibiotic substances from *Actinomyces* 27th Report. A new antifungal substance "Flavacid". Tohoku Jour. Exp. Med. [In press]. 1952.
206. TANNER, F. W., JR. et al. Thiolutin, an antibiotic produced by certain strains of *Streptomyces albus*. Abstracts of papers, 118th Meeting Am. Chem. Soc., Chicago, p. 18A. 1950.
207. ———— et al. Thiolutin, an antibiotic produced by certain strains of *Streptomyces albus*. Jour. Am. Chem. Soc. [In press].
207*a*. ———— et al. Some properties of Magnamycin, a new antibiotic. Antibiotics & Chemotherapy **2**: 441–443. 1952.
207*b*. TEJERA, E. et al. Mycobacidin: an antibiotic active against acid-fast organisms. Antibiotics & Chemotherapy **2**: 333. 1952.
208. TENER, G. M. [Personal communication].
209. THAYSEN, A. C. [Personal communication].
210. ———— and BUTLIN, K. R. Inhibition of the development of *Fusarium oxysporum cubense* by a growth substance produced by Meredith's actinomycete. Nature **156**: 781–782. 1945.
211. THORNE, C. B. and PETERSON, W. H. Xanthomycins A and B, new antibiotics produced by a species of *Streptomyces*. Jour. Biol. Chem. **176**: 413–428. 1948.
212. TOWNLEY, R. W. et al. Antibiotics produced by *Actinomyces vinaceus*. Chemical studies. Abstracts of papers, Twelfth International Congress of Pure and Applied Chem., page 284. 1951.
213. TRUSSELL, P. C. et al. Two antibiotics produced by a *Streptomyces*. Jour. Bact. **53**: 769–780. 1947.

214. ———— and RICHARDSON, E. M. Actinomycin from a new *Streptomyces*. Canad. Jour. Res., C., **26**: 27–30. 1948.
214*a*. UEDA, S. and UESAKA, I. On the antibiosis of *Nocardia*. Jour. Antibiotics [Japan] **5**: 170–171. 1952.
214*b*. UESAKA, I. Studies on the antibiotic action of *Nocardia*. I. Isolation and classification of antibiotic Actinomycetales. Jour. Antibiotics [Japan] **3**: 730–735. 1950.
214*c*. ————. Studies on the antibiotic action of *Nocardia*. II. Production of antibiotic substance by the surface culture of A.422 strain. Jour. Antibiotics [Japan] **3**(C): 27–34. 1950.
214*d*. ————. Studies on the antibiotic activity of *Nocardia*. III. Taxonomical studies of A.422 strain. Jour. Antibiotics [Japan] **5**: 75–79. 1952.
214*e*. ————. Studies on the antibiotic activity of *Nocardia*. IV. Extraction of the active substance from the fermented broth. Jour. Antibiotics [Japan] **5**: 154–159. 1952.
215. UMEZAWA, H. et al. On the various sources of actinomycin. Jour. Antibiotics [Japan] **4**: 335–338. 1951.
216. ———— et al. On a new antibiotic, griseolutein, produced by *Streptomyces*. Jour. Antibiotics [Japan] **4**: 34–40. 1951.
217. ———— et al. Studies on the antibacterial substance from a strain of *Actinomyces*. I. A crystalline antibacterial substance of a strain of *Actinomyces*. Jour. Penicillin [Japan] **1**: 129–133. 1947.
218. ———— et al. Isolation of a new antibiotic substance, aureothricin, from a strain of *Streptomyces*. Jap. Med. Jour. **1**: 512–517. 1948.
219. ———— et al. On the new source of chloromycetin, *Streptomyces omiyaensis*. Jap. Med. Jour. **2**: 207–211. 1949.
220. ———— et al. Isolation of a new antibiotic substance, aureothricin from a strain of *Streptomyces*. Jour. Antibiotics [Japan] **2-A**: 107–111. 1949.
221. ———— and TAKEUCHI, T. An antibiotic active to gram-positive bacteria, nitrosporin, from *Streptomyces nitrosporeus*. Jour. Antibiotics [Japan] **5**: 270–273. 1952.
222. ———— et al. An antiviral substance, abikoviromycin, produced by *Streptomyces* sp. Jap. Med. Jour. **4**: 331–346. 1951.
223. ———— et al. Studies on streptothricin BI produced by *S. fradiae*. Jour. Antibiotics [Japan] **3**: 833–838. 1950.
223*a*. UNO, S. [Personal communication].
224. VANDER BROOK, M. J. et al. Extraction and purification of streptomycin with a note on streptothricin. Jour. Biol. Chem. **165**: 463–468. 1946.
224*a*. VAN TAMELEN, E. E. and SMISSMAN, E. E. Streptolin. The structure and synthesis of isolysine. Jour. Am. Chem. Soc. **74**: 3713–3714. 1952.
225. VAUGHN, J. R. and HAMNER, C. L. The present status of cycloheximide (Acti-dione) as a fungicide. Proc. Am. Soc. Hort. Sci. **54**: 435–437. 1949.
226. ———— et al. The action of actidione on plant tissue and upon certain fungi. Mich. Agr. Exp. Sta. Quart. Bull. **31**: 456–464. 1949.
227. VINSON, J. W. and WALSH, F. T. The effect of terramycin on the PR8 strain of influenza A virus in chick embryos and mice. Jour. Immun. **65**: 129–134. 1950.
227*a*. WAKAKI, S. [Personal communication].
228. ———— et al. Antifungal substances produced by *Actinomyces*. II. An antibiotic from a strain resembling to *Streptomyces cacaoi*. I. Jour. Antibiotics [Japan] **5**: 24–29. 1952.
228*a*. ———— et al. Antifungal substances produced by *Actinomyces*. I. On the morphology of the antifungal-substance-producing strains. Jour. Antibiotics [Japan] **4**: 357–362. 1951.

229. WAKSMAN, S. A. The Actinomycetes. Their nature, occurrence, activities, and importance, p. 118. 1950.
229a. ————. Microbial antagonisms and antibiotic substances. 350 pp. 1945.
229b. ————. Streptomycin—isolation properties and utilization. Jour. Hist. Med. & Allied Sci. **3:** 318–347. 1951.
229c. ————. [Personal communication].
230. ———— et al. Micromonosporin, an antibiotic substance from a little-known group of microorganisms. Jour. Bact. **53:** 355–357. 1947.
231. ———— et al. Strain specificity and production of antibiotic substances. VII. Production of actinomycin by different actinomycetes. Proc. Nat. Acad. Sci. [U. S.] **32:** 117–120. 1946.
232. ———— et al. The distribution of antagonistic actinomycetes in nature. Soil Sci. **54:** 281–296. 1942.
233. ———— and LECHEVALIER, H. A. Neomycin, a new antibiotic active against streptomycin-resistant bacteria, including tuberculous organisms. Science **109:** 305–307. 1949.
234. ———— et al. Streptocin, antibiotic isolated from mycelium of *Streptomyces griseus*, active against *Trichomonas vaginalis* and certain bacteria. Proc. Soc. Exp. Biol. & Med. **70:** 308–312. 1949.
235. ———— et al. Studies on *Streptomyces lavendulae*. Jour. Bact. **62:** 149–161. 1951.
236. ———— et al. Toxicity of actinomycin. Proc. Soc. Exp. Biol. & Med. **47:** 261–263. 1941.
237. ———— and TISCHLER, M. Nature of actinomycin, an antimicrobial substance produced by *Actinomyces antibioticus*. Jour. Biol. Chem. **142:** 519–528. 1942.
238. ———— and WOODRUFF, H. B. Bacteriostatic and bactericidal substances produced by a soil *Actinomyces*. Proc. Soc. Exp. Biol. & Med. **45:** 609–614. 1940.
239. ———— and ————. *Actinomyces antibioticus*, a new soil organism antagonistic to pathogenic and non-pathogenic bacteria. Jour. Bact. **42:** 231–249. 1941.
240. ———— and ————. Streptothricin, a new selective bacteriostatic and bactericidal agent, particularly active against gram-negative bacteria. Proc. Soc. Exp. Biol. & Med. **49:** 207–210. 1942.
241. ———— et al. Actinomycetes as producers of antibiotics with special reference to the flavus group. Bact. Proc.: 30. 1951.
242. WALLACE, H. D. et al. Various antibiotics and 3-nitro-4-hydroxyphenyl arsonic acid in corn-peanut meal rations for swine. Proc. Soc. Exp. Biol. & Med. **78:** 807–808. 1951.
242a. WALLER, C. W. et al. Degradation of aureomycin. IV. Desdimethylamioaureomycinic acid. Jour. Am. Chem. Soc. **74:** 4979–4980. 1952.
242b. ———— et al. Degradation of aureomycin. VI. Isoaureomycin and aureomycin. Jour. Am. Chem. Soc. **74:** 4981. 1952.
242c. ———— et al. Degradation of aureomycin. VII. Aureomycin and anhydroaureomycin. Jour. Am. Chem. Soc. **74:** 4981–4982. 1952.
242d. WALTER, C. E. et al. [Personal communication from R. E. Hargrove].
243. WEBER, E. M. et al. Antibiotics as animal growth stimulants. Symp. Int. Res. Labs. Microbiol. World Health Organization, Rome, Italy. 1951.
244. WEISER, R. S. et al. Search for antibiotics active against *Mycobacterium tuberculosis*. 1. A streptothricin-like antibiotic from a *Streptomyces* sp. Proc. Soc. Exp. Biol. & Med. **72:** 283–287. 1949.
245. WELSCH, M. Bactericidal substances from sterile culture media and bacterial cultures, with special reference to the bacteriolytic properties of actinomycetes. Jour. Bact. **42:** 801–814. 1941.

246. ————. Bacteriostatic and bacteriolytic properties of actinomycetes. Jour. Bact. **44**: 571-588. 1942.
247. ————. Actinomycetin. Jour. Bact. **53**: 101–102. 1947.
247*a*. WELSH, H. Erythromycin—a new broad spectrum antibiotic. Antibiotics & Chemotherapy **2**: 279–280. 1952.
248. WHIFFEN, A. J. The production, assay, and antibiotic activity of actidione, an antibiotic from *Streptomyces griseus*. Jour. Bact. **56**: 283–291. 1948.
248*a*. ———— et al. The production of an antifungal antibiotic by *Streptomyces griseus*. Jour. Bact. **52**: 610–611. 1946.
249. WHITEHILL, A. R. et al. Stimulatory effect of aureomycin on the growth of chicks. Proc. Soc. Exp. Biol. & Med. **74**: 11–13. 1950.
250. WINTERSTEINER, O. and DUTCHER, J. D. Chemistry of antibiotics. Ann. Rev. Microbiol. **3**: 559–594. 1949.
251. WOODRUFF, H. B. and FOSTER, J. W. Streptin, an antibiotic from a species of *Streptomyces*. Jour. Bact. **52**: 502. 1946.
251*a*. WORK, T. S. The biochemistry of antibiotics. Ann. Rev. Biochem. **21**: 431–458. 1952.
251*b*. YACOWITZ, H. et al. The distribution of aureomycin, Chloromycetin, procaine penicillin G, and terramycin in the gastrointestinal tract of the chick. Informal Poultry Nutrition Conference, New York, 1952.

Future Contents of

THE BOTANICAL REVIEW

Articles Received and Awaiting Publication

Wood Fiber Length as Related to Position in Tree and Growth	Stephen H. Spurr *University of Minnesota*
Compression Wood in Conifers as a Morphogenetic Phenomenon	
Nitrogen Sources of Seed Plants and Related Environmental Influences	Lora Mangum Shields *New Mexico Highlands University*
Decomposition of Cellulose by Microorganisms	R. G. H. Siu and E. T. Reese *U. S. Army Quartermaster Corps*
Seed Germination Problems in the Umbelliferae	Richard Robinson *University of California*
Suggestions for the Classification and Nomenclature of Fossil Spores and Pollen Grains	Divya Darshan Pant *University of Allahabad*
Comparative Physiology of Graminaceous Plants in Relation to Nitrogen	K. N. Lal and M. S. Subba Rao *Banaras Hindu University*
The Telome Theory	Carl L. Wilson *Dartmouth College*
The Cytogenetics of Triticale	Joseph G. O'Mara *Iowa Agr. Exp. Sta.*
The Structure and Behavior of Fungous Nuclei	Lindsay S. Olive *Columbia University*
Disease Resistance in the Vegetable Crops	J. C. Walker *University of Wisconsin*
Heart Rots in Living Trees	W. W. Wagener and R. W. Davidson *U. S. Dept. of Agriculture*

Outstanding McGRAW-HILL *Books*

PLANT MORPHOLOGY

By ARTHUR W. HAUPT, University of California, Los Angeles. *McGraw-Hill Publications in the Botanical Sciences.* Ready for fall classes.

A text for upper-division botany majors, this book treats the principal plant groups in relation to structure, reproduction, and development. In a survey of the plant kingdom, emphasis is upon relationships seen in similarities of bodily organization and life histories. Presenting an evolutionary account, it interprets complexities of higher plants in terms of simpler lower plants, evolutionary tendencies within groups, and interrelationships.

BOTANY. A Textbook for Colleges.

By J. BEN HILL, The Pennsylvania State College, LEE O. OVERHOLTS; and HENRY W. POPP, The Pennsylvania State College. *McGraw-Hill Publications in the Botanical Sciences.* Second Edition, 690 pages, $6.00.

This revised edition treats recent advances in the science in photosynthesis, respiration, fermentation and enzymes, metabolism, hormones and vitamins, antibiotics, the use of "tagged elements", hydroponics, etc. New sections have been added on viruses, actinomycetes, diatoms, the economic importance of fungi as well as a special section on meiosis which is probably unique in general textbooks of botany.

AN INTRODUCTION TO THE EMBRYOLOGY OF ANGIOSPERMS

By P. MAHESHWARI, University of Delhi. *McGraw-Hill Publications in Botanical Sciences.* 439 pages, $7.00.

Gives an up-to-date account of the embryology of the angiosperms in the widest sense, covering not only the embryo and endosperm, but also the development of the male and female gametophytes and the process of fertilization. The author shows that modern embryology is not a mere narration of facts but a rapidly advancing science having many contacts with other fields of study.

THE FRESH-WATER ALGAE OF THE UNITED STATES

By GILBERT M. SMITH, Stanford University. *McGraw-Hill Publications in the Botanical Sciences.* 719 pages, $11.00.

This standard reference and advanced text enables students to identify the fresh-water algae of this country and to summarize the morphology and life histories. The book includes the choras, cryptomonads, and chloromonads. For each genus, references are given to descriptions of the species found in the United States. Each of the new genera is illustrated.

Send for copies on approval

McGRAW-HILL BOOK COMPANY, INC.

330 WEST 42ND STREET, NEW YORK 18, N. Y.